THE USE OF CUTICULAR FEATURES IN THE TAXONOMY OF THE LAURACEAE[1]

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ABSTRACT

The Lauraceae, with more than 2500 species in over 50 genera, and with the general lack of agreement in their recent classification, make an ideal candidate for a global study based on leaf cuticles. A technique for preparing cuticles is presented, and three types of characters of use in such studies (cell form, stomatal complex, and special cell types) are discussed. Preliminary results demonstrate the utility of these cuticular characters in defining genera such as *Endiandra* and *Beilschmiedia* and in sorting out biogeographical anomalies, such as found in *Caryodaphnopsis*, where the cuticles confirm placement of disjunct species within one genus. Potential challenges/problems posed by larger genera such as *Litsea*, *Cryptocarya*, *Cinnamomum*, *Ocotea*, and *Nectandra* are discussed.

Two of the most recent classifications of the Lauraceae are those of Kostermans (1957) and Rohwer (Kubitzki et al., 1993). The former is based on his own life-long studies, and the latter relies heavily on Kostermans and on the wood anatomical studies of Richter (1981), as well as including studies (primarily South American) by the author himself. The problems associated with these and other classifications of the Lauraceae are discussed by van der Werff and Richter (1996, this issue). From that review it is clear that further work is required to arrive at a satisfactory, or at least widely acceptable, classification of the family. Cuticular, and to a smaller degree, leaf architectural studies form one module in a multifaceted study of the generic delimitations of this family currently in progress.

The aim of this study is to analyze cuticular features for selected Lauraceae species whose generic positions have been predetermined by others, and in some cases questioned. At this time, no attempt has been made to use these features to confirm or disprove the evolutionary position, or indeed the "monophyly" (sensu cladistics), of these groups of Lauraceae. Consistent with that, individual features are not weighted or equalized, but are simply examined in light of the hypotheses generated by the placement of the species in genera by earlier workers. Characters that have shown consistency in this study are being included by the first author in the global generic revision of the Lauraceae, which follows on from this project. Even in that study, the primary aim will be to place groups of species in consistently recognizable genera, and not necessarily to propose phylogenetic relationships (cladistic or otherwise).

Cuticular studies of the Lauraceae are not novel. As early as 1926, Bandulska presented a study of fossil and extant Lauraceae and described some species of the former based on cuticular similarities to certain extant species. More recently, Hill (1986) discussed those features of the cuticle that allowed identification of Lauraceae, and placed the 12 species of fossil Lauraceae found in the Eocene Nerriga deposit within the form genus *Laurophyllum* based on these cuticular features. He made no attempt to place them relative to extant genera. Christophel and Rowett (in press) present descriptions of leaf architecture and cuticles of all species of leafy Lauraceae found in Australia. They also present a dichotomous key to cuticular features that allows identification to the generic (or to a species group within genus) level.

While most of the genera occurring in Australia can be clearly defined (extant leafy genera found there include *Beilschmiedia*, *Cinnamomum*, *Cryptocarya*, *Endiandra*, *Lindera*, *Litsea*, and *Neolitsea*), a notable exception is *Cryptocarya*. Although its species do not intermingle with those of any other

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genus, no set of characters has been found that holds all species together and yet separates the genus from others (Christophel & Rowett, in press). The result is that five groups of species key out to *Cryptocarya* in different parts of the key. Unfortunately, these five groupings do not correspond to those of Hyland (1989). This discrepancy indicates that further study of *Cryptocarya* is warranted, with the possibility existing that the genus as now defined is not natural and perhaps even polyphyletic. The heterogeneity, and perhaps artificiality of this large genus, has been previously suggested based on other features (e.g., Rohwer in Kubitzki, 1993).

In both the systems of Kostermans (1957) and of Rohwer (1993), the genera occurring in Australia are well spread across the major subfamilial groupings, and hence the mainly successful generic separations of Christophel and Rowett (in press) suggest that their character set represents a good starting place for the global generic revision. Dichotomous keys in general (e.g., Christophel & Rowett, in press) suffer from the requirement that certain features must be used before other (perhaps more obvious) ones can be applied. Therefore, the results of the global revision will be presented as a computerized, multi-entry key. If genera such as *Cryptocarya* do not fall out more reasonably when a better sample of their species is included, then the polyphyletic nature of the genus will be supported.

METHODS

The majority of the leaf samples used in this and the ensuing complete study have been provided, or at least have had their identifications confirmed, by either H. van der Werff, Missouri Botanical Garden (MO) or B. P. M. Hyland, Herbarium Australiense–Atherton (QRS), who are both participating in the global revision of the Lauraceae. Hence the consistency of identification is maximized. Samples were only taken from mature leaves, and 1-cm^2 pieces were taken from the near basal margin on the lefthand side of the leaf with the adaxial surface upward, to eliminate variation that might have been caused by sampling from different areas. At least three samples from two different collections were used whenever possible, although for some species only one collection could be obtained, in which case several leaves from it were used. A list of specimens studied is presented in Table 1.

While the technique for cuticle preparation is similar to that used by Christophel and Rowett (in press), it is described here for completeness. The pieces of leaf are placed in small test tubes and soaked in 70–95% ethanol for approximately 18 hours. Experiments have shown that no observable difference in either quality of preparation or speed can be detected in samples by varying the ethanol strength within the above range. The ethanol is then decanted off and 10 drops of 40% w/vol H_2O_2 and five drops of 90% ethanol are added to the test tube, which is then heated to gentle boiling in a water bath for 2–48 hours. The cooking can be judged complete when the sample has turned light yellow to white and when the cut edges of the cuticle can be observed to be peeling back from the leaf surface like the two covers of a book. At that point the sample can be removed from the test tube and placed in a watch glass or petri dish of water, where the "book covers" are pried open and the "pages" of the book, or the actual cellular material, can be brushed away with fine artists' brushes. If removal of cellular contents specimens at this stage proves difficult, the specimens can be returned to 70–90% ethanol for a further 12 hours, which usually renders them much easier to clean.

While no chemical reaction involving the ethanol and H_2O_2 is apparent, leaf pieces pre-soaked in this manner clear between three and four times faster than those not pretreated. It may be that the ethanol acts to break surface tension on the cells, thus facilitating entrance of the hydrogen peroxide into the tissue. This is supported by the fact that preparation of fresh leaf material, which is naturally hydrated, is not enhanced to the same degree by the pretreatment in ethanol as are dried specimens.

Cleaned cuticles can be quickly rinsed in 2% ammonia to adjust pH, and then stained in 0.1% Crystal Violet for 20–120 seconds, or until the cuticle appears uniformly light purple to the naked eye. Under the compound microscope, anticlinal cell walls should appear bright to dark purple, as should thickened cuticular ledges, but the lumens of the cells should not appear stained, or with only a slight tinge of purple. Overstained samples can be destained by rinsing in 90–100% ethanol. In general, understained slides are preferable to overstained ones, as various techniques, including interference contrast, can be used to compensate for lack of stain, but little can be done to observe obscured detail in overstained cuticles.

Stained cuticles can be mounted in phenol glycerin jelly. When the slides have set and excess jelly is cleaned from the coverslip with warm water and a razor blade, the coverslip can be ringed with nail varnish (polish) to retard dehydration. If more permanent mounting is desired, the stained cuticles can be dehydrated and mounted in a resin-based medium. However, the first author has had slides

Table 1. Specimens examined.

Species	Country	Collector & number
Aiouea guianensis Aublet	Brazil	G. T. Prance 4401
Aiouea guianensis Aublet	Guiana	Jansen Jacobs 1986
Beilschmiedia bancroftii (Bailey) C. White	Australia	B. Gray 3787
Beilschmiedia bancroftii (Bailey) C. White	Australia	B. Gray 3977
B. brunnea B. Hyland	Australia	B. Gray 3900
B. brunnea B. Hyland	Australia	B. Gray 1837
B. castrisinensis B. Hyland	Australia	B. Gray 1092
B. castrisinensis B. Hyland	Australia	B. Gray 3210
B. castrisinensis B. Hyland	Australia	B. Gray 4158
B. collina B. Hyland	Australia	B. Hyland 10104
B. collina B. Hyland	Australia	B. Gray 1605
B. elliptica C. White & Francis	Australia	B. Hyland 4128
B. obtusifolia (F. Muell. ex Meissner) F. Muell.	Australia	B. Gray 1279
B. obtusifolia (F. Muell. ex Meissner) F. Muell.	Australia	B. Gray 3978
B. oligandra L. S. Smith	Australia	B. Gray 595
B. oligandra L. S. Smith	Australia	B. Gray 1726
B. oligandra L. S. Smith	Australia	B. Gray 2786
B. peninsularis B. Hyland	Australia	B. Hyland 11572
B. peninsularis B. Hyland	Australia	B. Gray 3623
B. recurva B. Hyland	Australia	B. Gray 276
B. recurva B. Hyland	Australia	B. Gray 4201
B. tooram (Bailey) B. Hyland	Australia	B. Gray 2826
B. tooram (Bailey) B. Hyland	Australia	B. Gray 3700
B. volckii B. Hyland	Australia	B. Gray 3823
B. volckii B. Hyland	Australia	B. Gray 2923
Caryodaphnopsis henrii A. Shaw	China	Kunming Herb. 645874
C. tonkinensis (Lec.) A. Shaw	China	Kunming Herb. 0125
C. tonkinensis (Lec.) A. Shaw	Philippines	Ramos 1667
C. sp. nov.	Peru	Jaramillo 11930
Chlorocardium venenosum Rohwer et al.	Colombia	Cogollo 5153
Chlorocardium venenosum Rohwer et al.	Ecuador	Zak 3913
Cinnamomum baileyanum (F. Muell. ex Bailey) Francis	Australia	B. Hyland 2648
C. laubatii F. Muell.	Australia	B. Gray 315
C. laubatii F. Muell.	Australia	B. Gray 3923
C. laubatii F. Muell.	Australia	B. Gray 3925
C. oliveri Bailey	Australia	B. Hyland 3303
C. oliveri Bailey	Australia	B. Hyland 12456
C. propinquum Bailey	Australia	B. Gray 686
C. propinquum Bailey	Australia	B. Gray 921
C. sellovianum (Nees & Mart.) Kosterm.	Brazil	Harley 25261
C. sellovianum (Nees & Mart.) Kosterm.	Brazil	Duarte 9295
C. virens R. Baker	Australia	B. Hyland 12340
Cryptocarya sp.	Madagascar	Miller 3725
Cryptocarya sp.	Madagascar	Miller 3955
Cryptocarya sp.	Madagascar	Armbruster 3145
Endiandra acuminata C. White & Francis	Australia	B. Gray 1368
Endiandra acuminata C. White & Francis	Australia	B. Gray 1583
Endiandra acuminata C. White & Francis	Australia	B. Gray 2332
E. anthropophagoram Domin.	Australia	B. Hyland 11712
E. anthropophagoram Domin.	Australia	B. Gray 2951
E. bellendenkerana B. Hyland	Australia	B. Gray 2953
E. bellendenkerana B. Hyland	Australia	B. Gray 2072
E. bessaphila B. Hyland	Australia	B. Gray 1644
E. bessaphila B. Hyland	Australia	B. Hyland 10650
E. collinsii B. Hyland	Australia	B. Hyland 3570
E. collinsii B. Hyland	Australia	B. Hyland 11134

Table 1. Continued.

Species	Country	Collector & number
E. compressa C. White	Australia	B. Gray 1753
E. compressa C. White	Australia	B. Gray 3723
E. cooperana	Australia	B. Gray 3209
E. cooperana	Australia	B. Gray 3565
E. cowleyana	Australia	B. Gray 1818
E. cowleyana	Australia	B. Gray 4123
E. crassiflora C. White & Francis	Australia	B. L. Jones 1283
E. dichrophylla F. Muell.	Australia	B. Gray 2915
E. dichrophylla F. Muell.	Australia	B. Gray 3713
E. dielsiana Teschner	Australia	B. Hyland 13176
E. dielsiana Teschner	Australia	B. Gray 3890
E. discolor Benth.	Australia	B. Hyland 2996
E. discolor Benth.	Australia	B. Gray 3012
E. floydii B. Hyland	Australia	B. Hyland 4619
E. glauca R. Br.	Australia	B. Gray 3211
E. glauca R. Br.	Australia	B. Hyland 14039
E. globosa Maiden & E. Betche	Australia	B. Gray 3173
E. globosa Maiden & E. Betche	Australia	B. Gray 4152
E. grayi B. Hyland	Australia	B. Gray 3157
E. grayi B. Hyland	Australia	B. Gray 3726
E. grayi B. Hyland	Australia	B. Gray 4084
E. grayi B. Hyland	Australia	B. Gray 4162
E. hayesii Kosterm.	Australia	B. Hyland 4553
E. hypotephra F. Muell.	Australia	B. Gray 456
E. hypotephra F. Muell.	Australia	B. Gray 3969
E. hypotephra F. Muell.	Australia	B. Gray 4001
E. impressicosta Alenn	Australia	B. Gray 2454
E. impressicosta Alenn	Australia	B. Gray 3101
E. insignis (Bailey) Bailey	Australia	B. Gray 1641
E. insignis (Bailey) Bailey	Australia	B. Gray 3714
E. introrsa C. White	Australia	B. Hyland 12338
E. jonesii	Australia	B. Gray 2255
E. jonesii	Australia	B. Gray 2869
E. kingiana Gamble	Sarawak	B. Hyland 14146
E. leptodendron B. Hyland	Australia	B. Gray 3924
E. leptodendron B. Hyland	Australia	B. Gray 3953
E. limnophila B. Hyland	Australia	B. Hyland 12375
E. limnophila B. Hyland	Australia	B. Hyland 12377
E. longipedicellata C. White & Francis	Australia	B. Hyland 6256
E. longipedicellata C. White & Francis	Australia	B. Gray 3243
E. microneura C. White	Australia	B. Gray 3822
E. monothyra B. Hyland	Australia	B. Hyland 12584
E. monothyra B. Hyland	Australia	B. Gray 1647
E. monothyra B. Hyland	Australia	B. Gray 1652
E. monothyra B. Hyland	Australia	B. Gray 3284
E. monothyra B. Hyland	Australia	B. Gray 3673
E. montana C. White	Australia	B. Gray 2825
E. montana C. White	Australia	B. Gray 3127
E. muelleri Meissner in DC	Australia	B. Hyland 12330
E. muelleri Meissner in DC	Australia	B. Hyland 12957
E. muelleri Meissner in DC	Australia	B. Hyland 12972
E. palmerstonii (Bailey) C. White	Australia	B. Hyland 11721
E. phaeocarpa B. Hyland	Australia	B. Gray 3297
E. phaeocarpa B. Hyland	Australia	B. Gray 3873
E. pubens Meissner in DC	Australia	B. Gray 2971
E. sankeyana Bailey	Australia	B. Gray 3579
E. sankeyana Bailey	Australia	B. Gray 4103

Table 1. Continued.

Species	Country	Collector & number
E. sideroxylon B. Hyland	Australia	B. Gray 3904
E. sideroxylon B. Hyland	Australia	B. Gray 4148
E. sieberi Nees	Australia	B. Gray 1504
E. virens F. Muell.	Australia	B. Hyland 4602
E. wolfii B. Hyland	Australia	B. Gray 1328
E. wolfii B. Hyland	Australia	B. Gray 3962
E. xanthocarpa B. Hyland	Australia	B. Gray 1614
E. xanthocarpa B. Hyland	Australia	B. Gray 5155
E. sp.	Java	B. Hyland 14217
Litsea costalis (Nees) Kosterm.	Sembilon	B. Hyland 14130
Ocotea lentii Burger	Costa Rica	Moraga 171
Ravensara sp.	Madagascar	Miller 3835
Ravensara sp.	Madagascar	G. McPherson 14428
Rhodostemonodaphne grandis (Mez) Rohwer	French Guiana	Mori 20904
Rhodostemonodaphne grandis (Mez) Rohwer	Peru	Kroll 134
Rhodostemonodaphne grandis (Mez) Rohwer	Peru	Kayap 3541
R. kunthiana (Nees) Rohwer	Ecuador	Zak 4452
R. kunthiana (Nees) Rohwer	Costa Rica	Herrera 2397

mounted in glycerin jelly for 20 years without harm befalling them, and the advantages of that medium are that it is inexpensive, quick to use, and the cuticles can be retrieved by soaking the slide in hot water if other preparations or stain adjustments are to be made later. For long-term storage in a dry environment, dehydration will be better retarded by ringing slides in a wax. Safranin staining also provides good visual detail of the cuticles, but the red color necessitates the use of a green filter for maximum contrast with black-and-white photography, while the purple staining of Crystal Violet does not require this.

All cuticular photographs (Figs. 1–18, 20–25) were taken using a Zeiss automatic 35mm camera on a Zeiss Axioplan microscope with a 40× objective and 10× eyepiece.

CUTICULAR CHARACTERS

Leaf cuticular characters used to delimit genera in the Australian Lauraceae (Christophel & Rowett, in press) fall into three broad categories: (1) those of the walls of normal, non-stomatal cells, (2) those of the stomatal complex (including subsidiary cells), and (3) those of specialized cells such as trichomes, trichome bases, and hydathodes. The Lauraceae appear to be uniformly hypostomatic, and hence separate character sets can be used for the adaxial (upper) and abaxial (lower) cuticles. In practice, leaves of many species of Lauraceae appear to differ in their two cuticular surfaces, not only in presence/absence of stomata, but also in features of the indument and of general epidermal cell size and shape (e.g., Figs. 1, 2).

Considering first the features of non-stomatal cells, the periclinal walls can be seen to vary from densely papillose (Fig. 1) to lightly papillose (Fig. 3), granular (Fig. 2), punctate (Fig. 10), slightly striate (Fig. 6), or smooth (Figs. 14, 18). To be considered granular, the periclinal walls must be regularly and coarsely so. There are probably several subtly different states between coarsely granular

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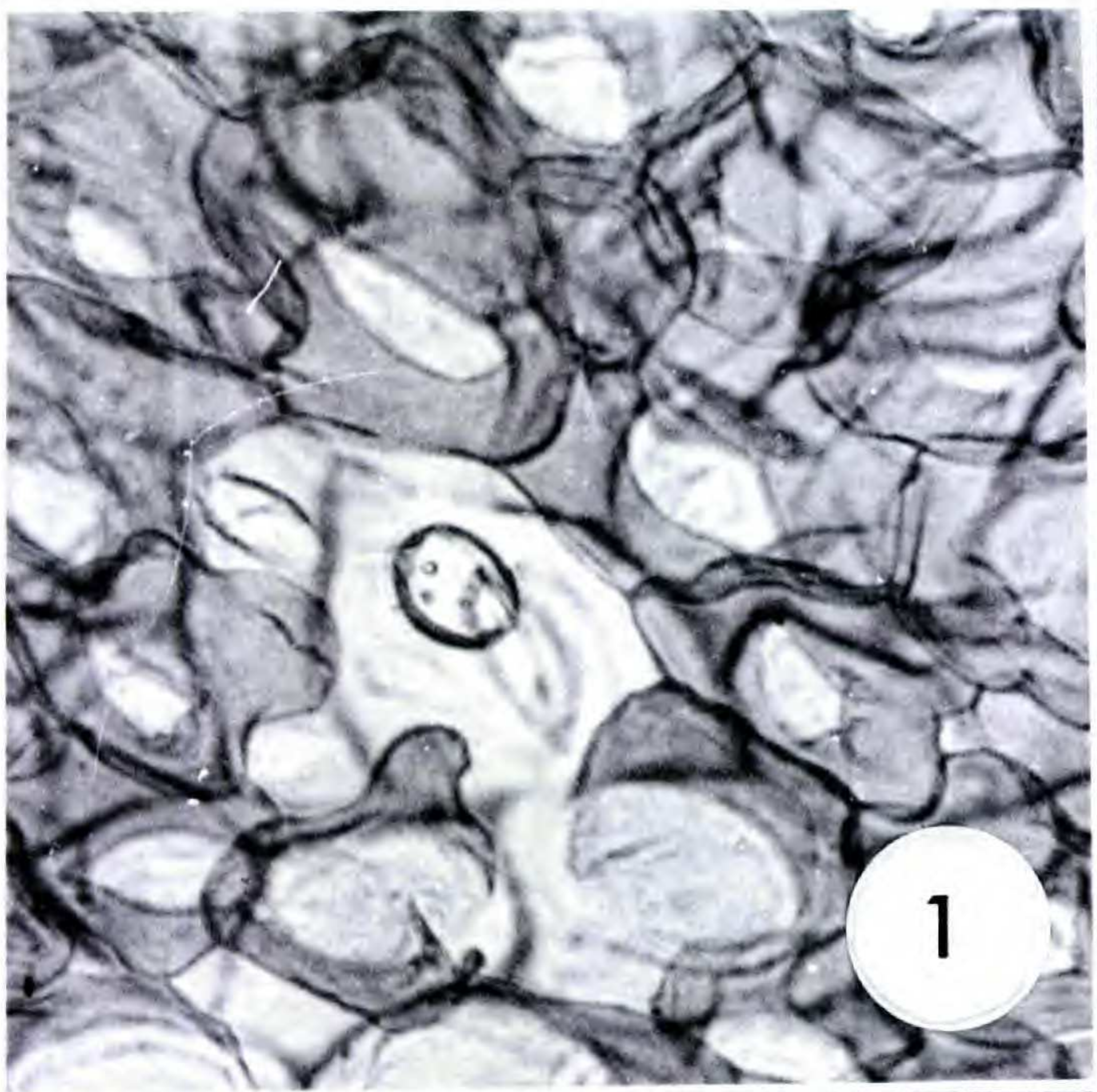
Figures 1–6. —1. Abaxial cuticle of *Caryodaphnopsis henrii* A. Shaw, from L509, Botany Department Herbarium, Adelaide University; collected from the Kunming Botanical Institute Herbarium (#645874), Kunming, China. Collector and number in Chinese and not readable. Collection from Southern China. Magnification as per Figure 24.—2. Adaxial cuticle of *Caryodaphnopsis henrii* A. Shaw. All information and magnification as per Figure 24.—3. Abaxial cuticle of *Caryodaphnopsis* sp. nov. taken from L317, Botany Department Herbarium, Adelaide University, from *Jaramillo 11930* at MO, from Peru; magnification as per Figure 24.—4. Abaxial cuticle of *Caryodaphnopsis tonkinensis* (Lec.) A. Shaw from L508, Botany Department Herbarium, Adelaide University, from the Botanical Institute of Kunming, China, (sheet #0125); collected in southern China; magnification as per Figure 24.—5. Abaxial cuticle of *Chlorocardium venenosum* Rohwer, Richter & van der Werff taken from L398, Botany Department Herbarium, Adelaide University; originally from *Cogollo 5153* at MO; collected in Colombia. Scale bar as per Figure 20.—6. Adaxial cuticle of *Chlorocardium venenosum*. All details and magnification as per Figure 5.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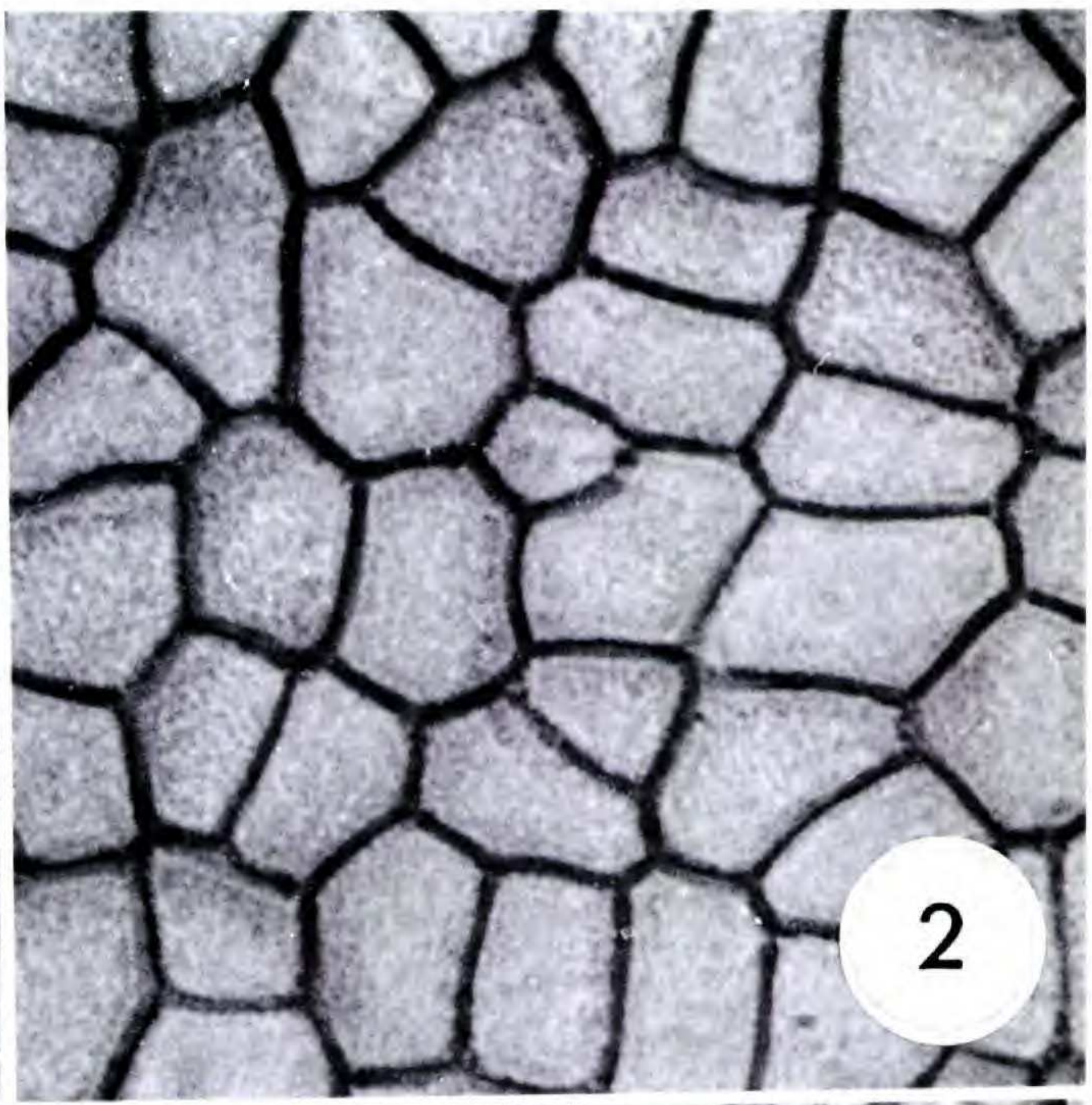
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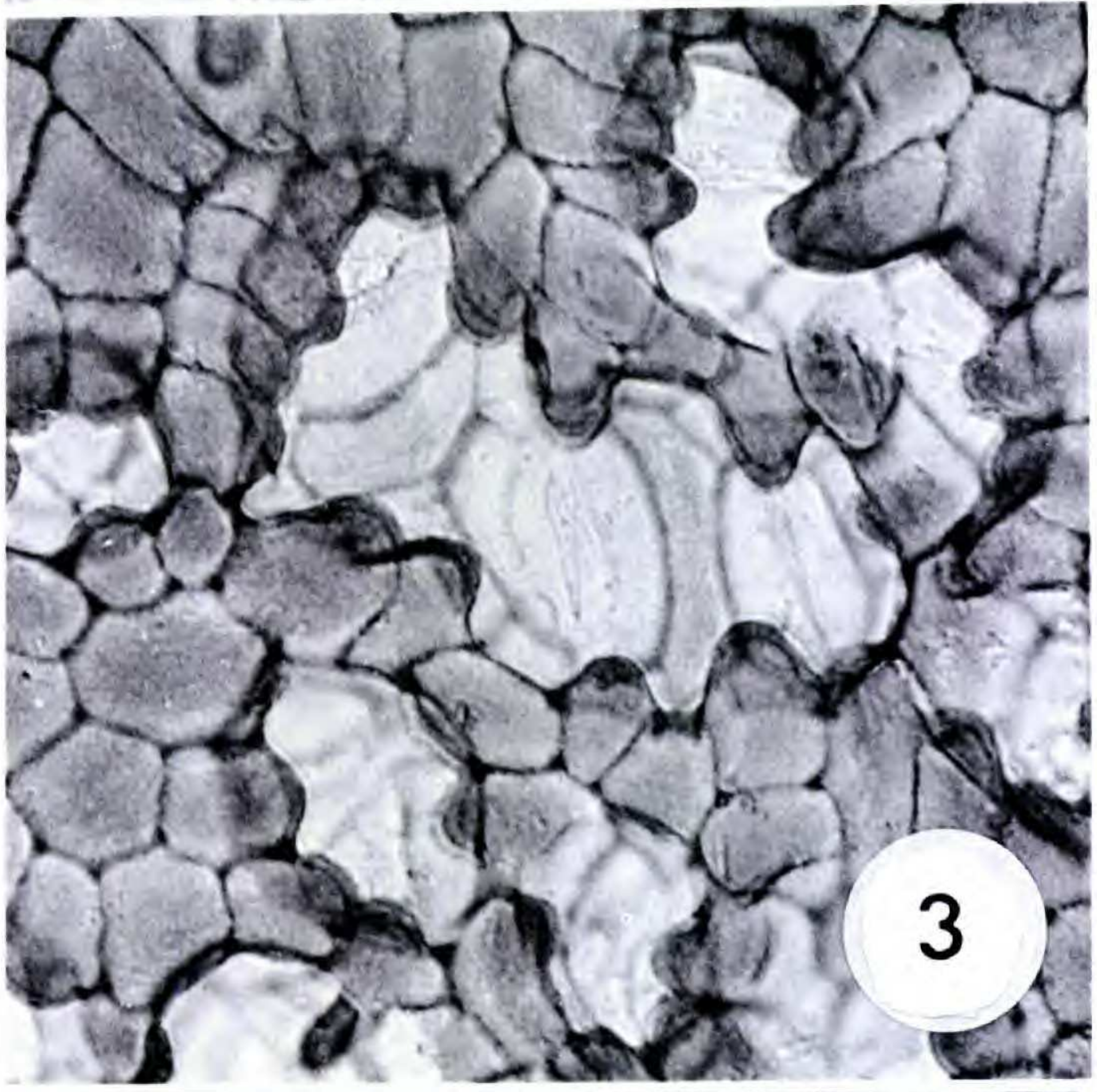
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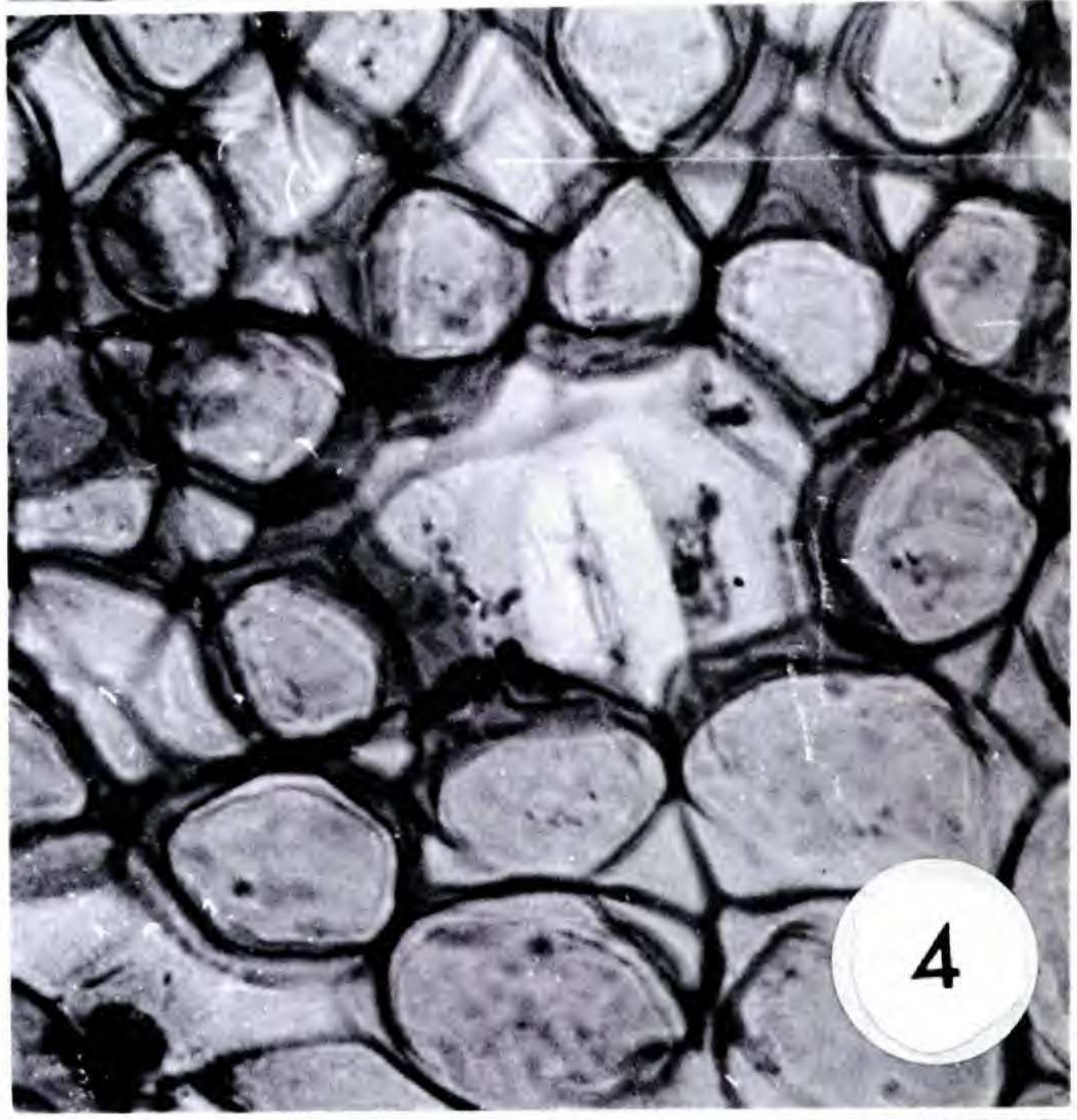
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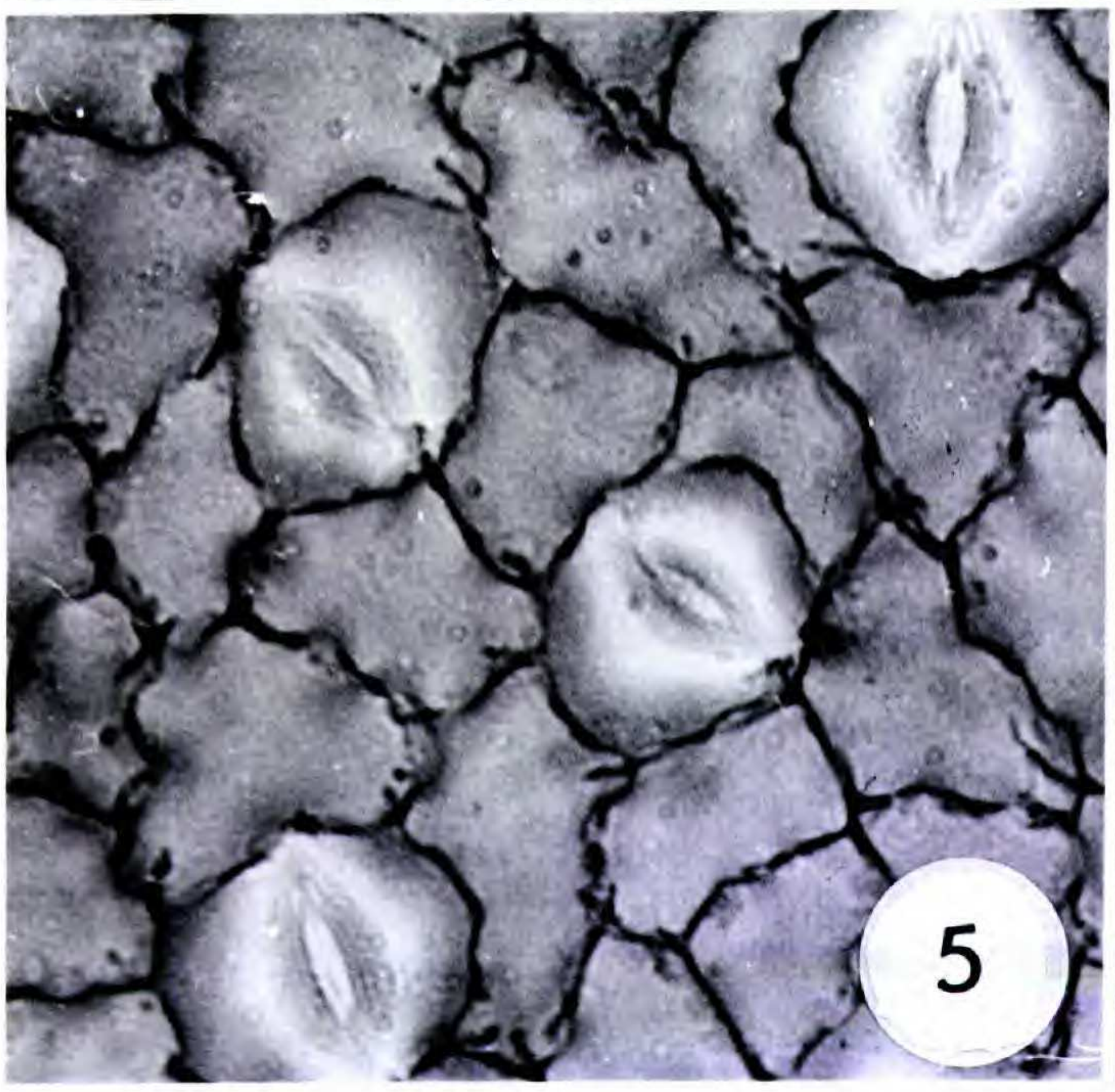
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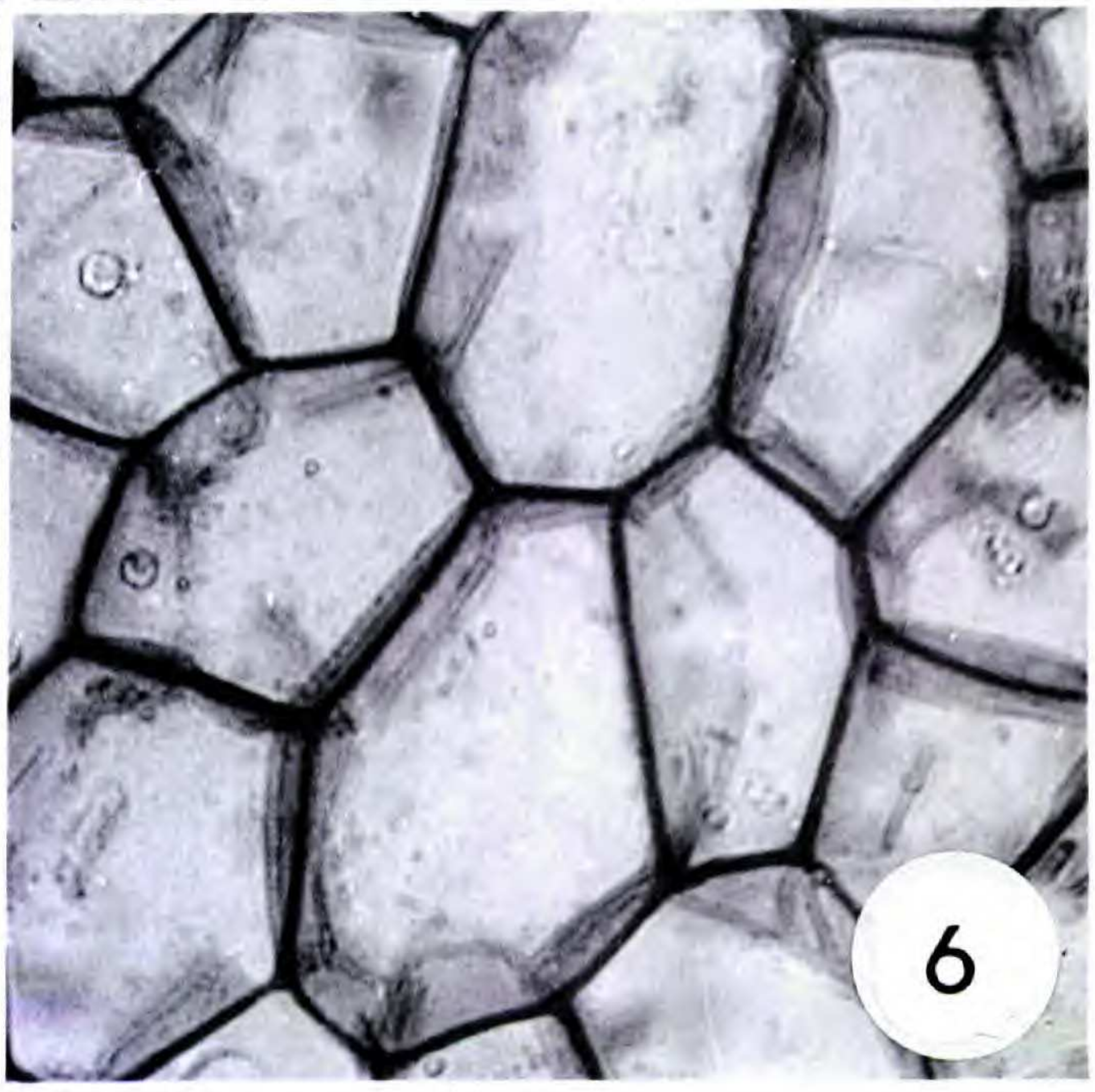
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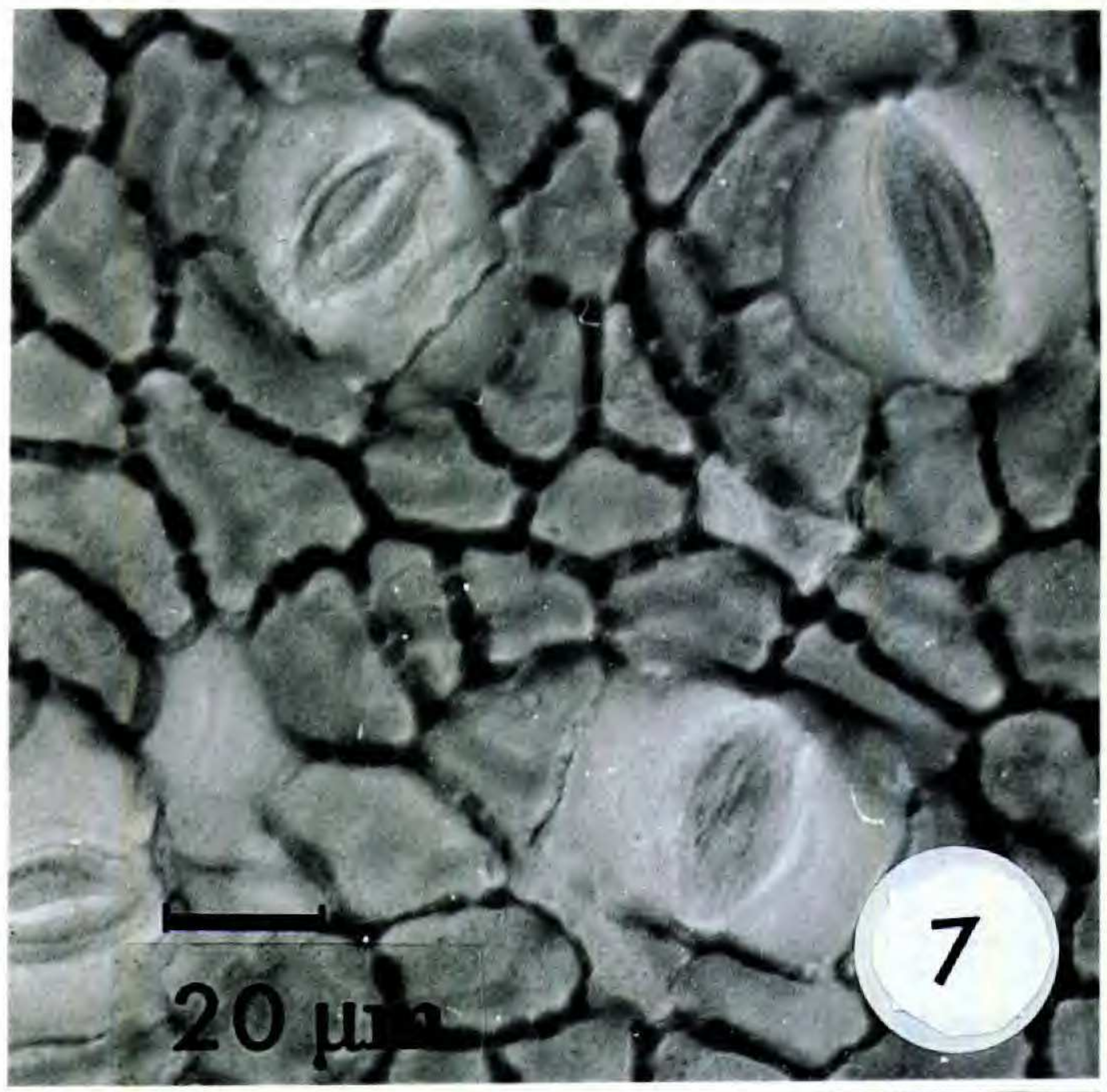

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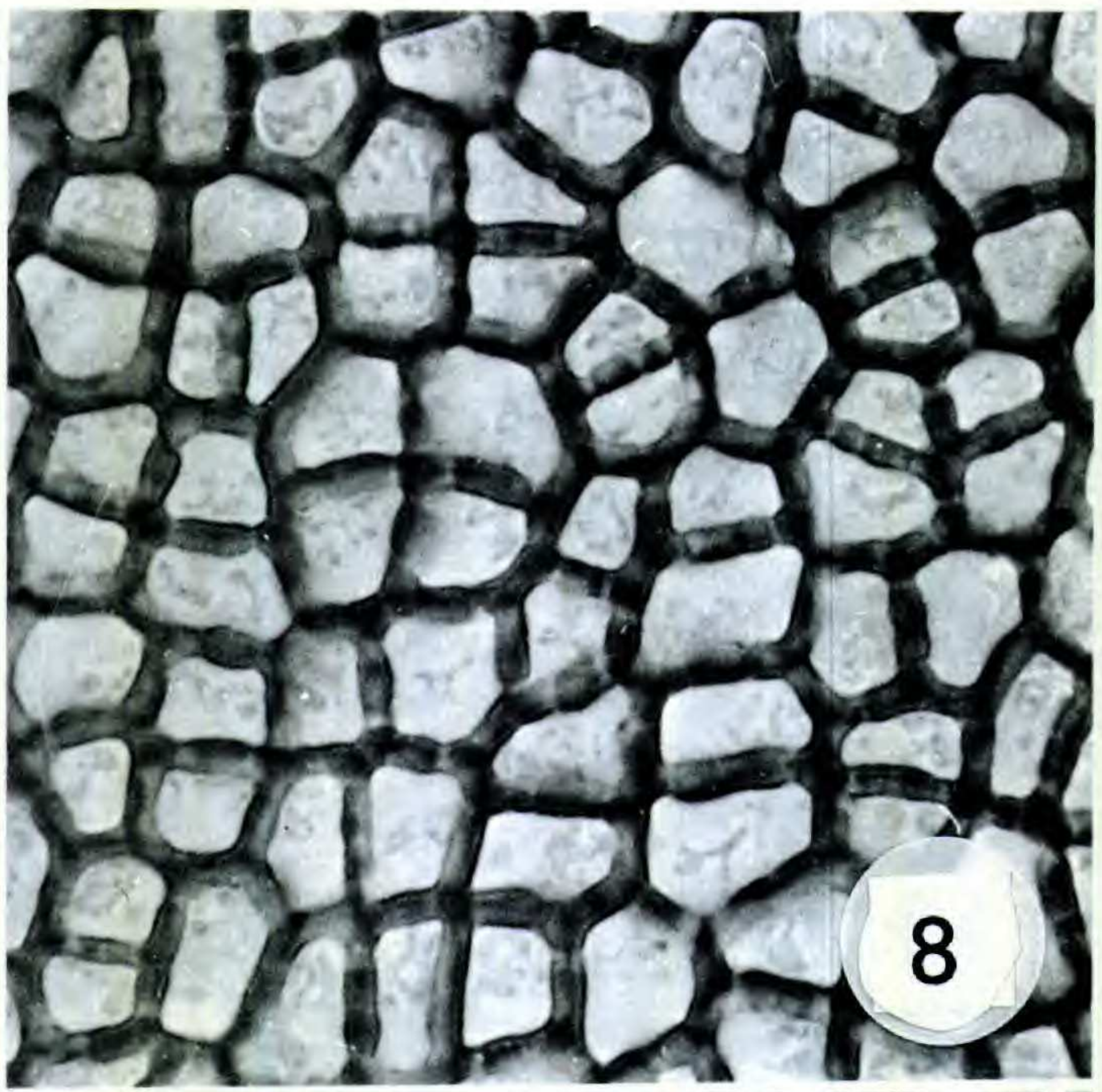

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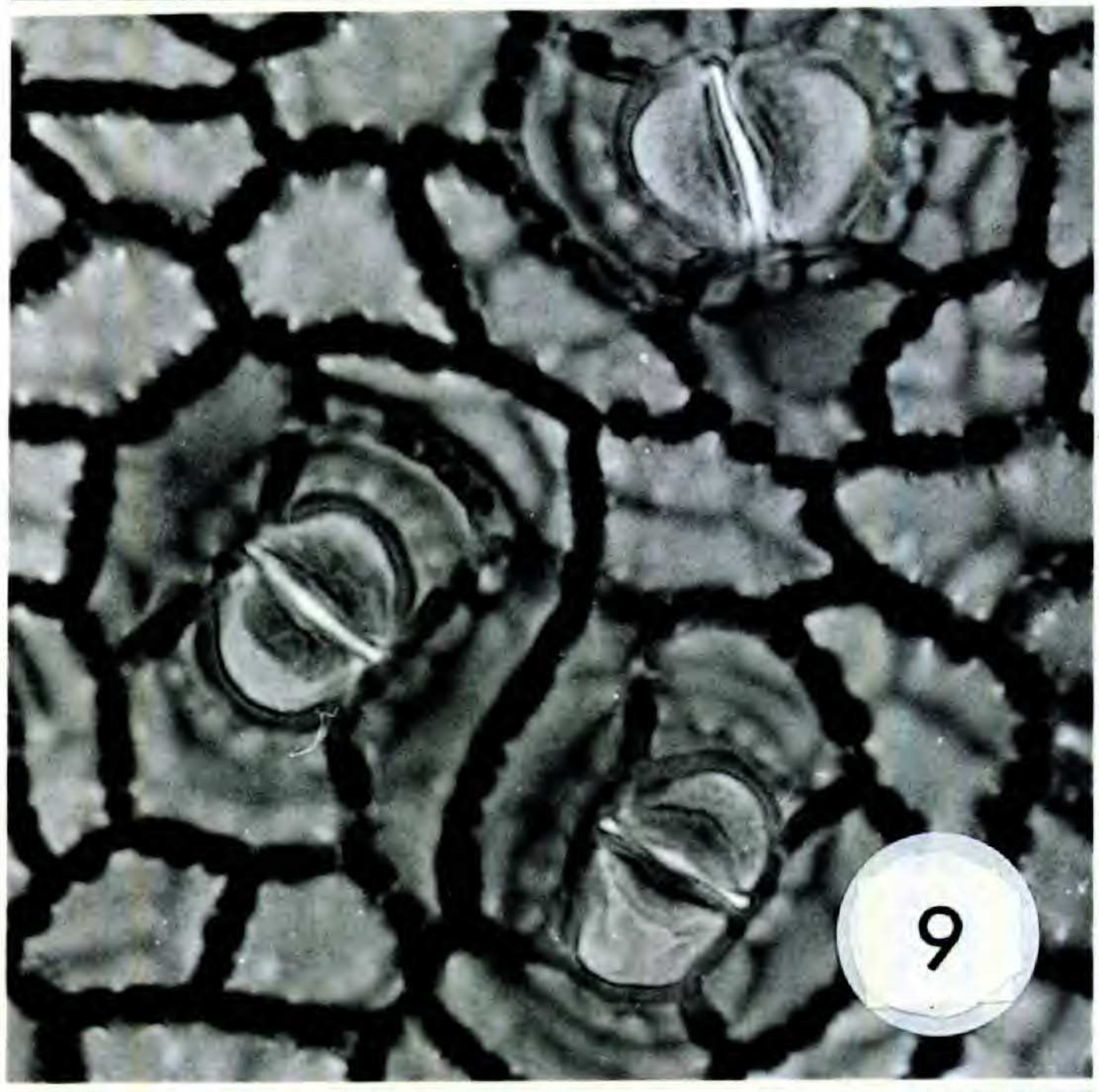

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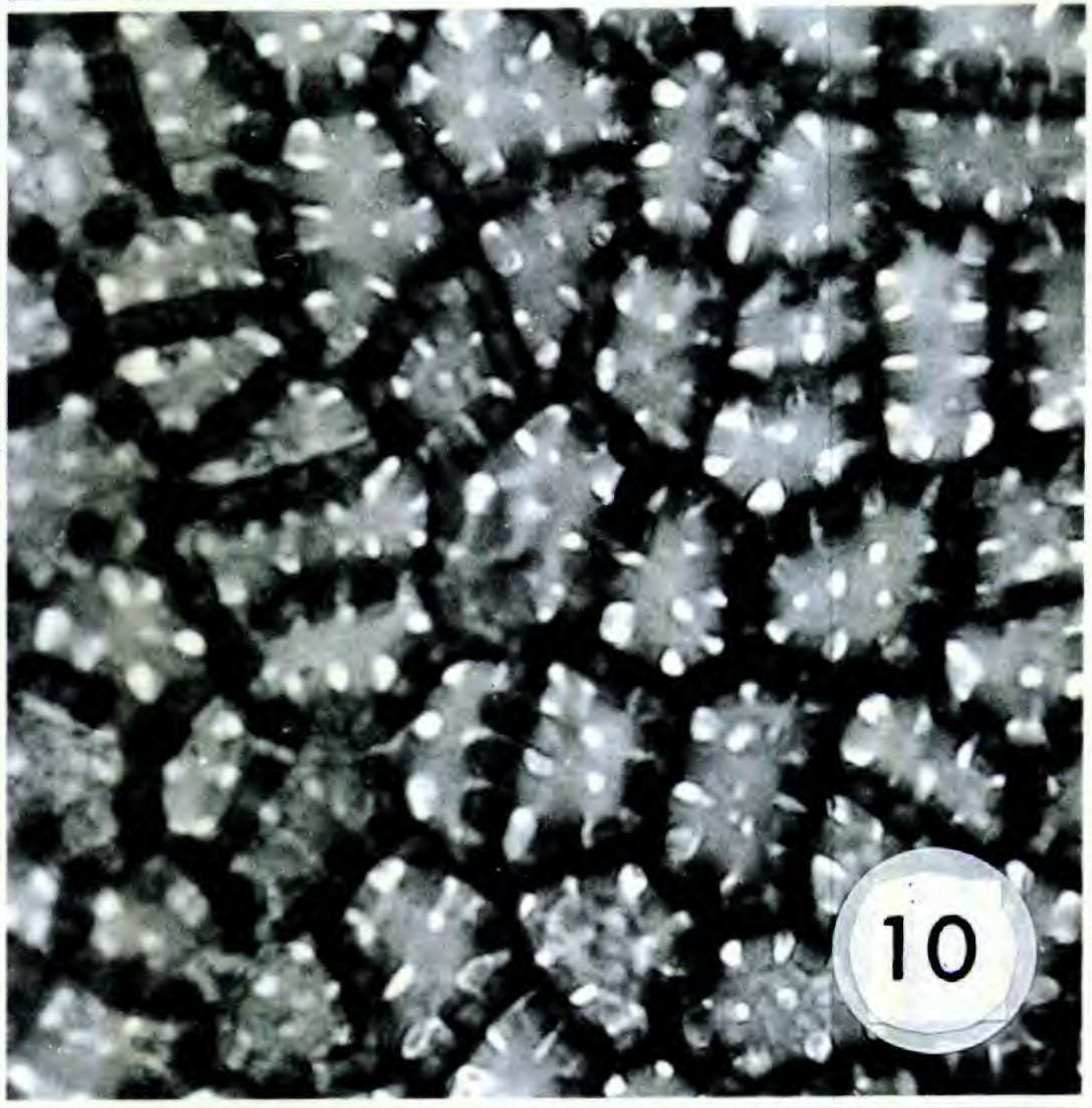

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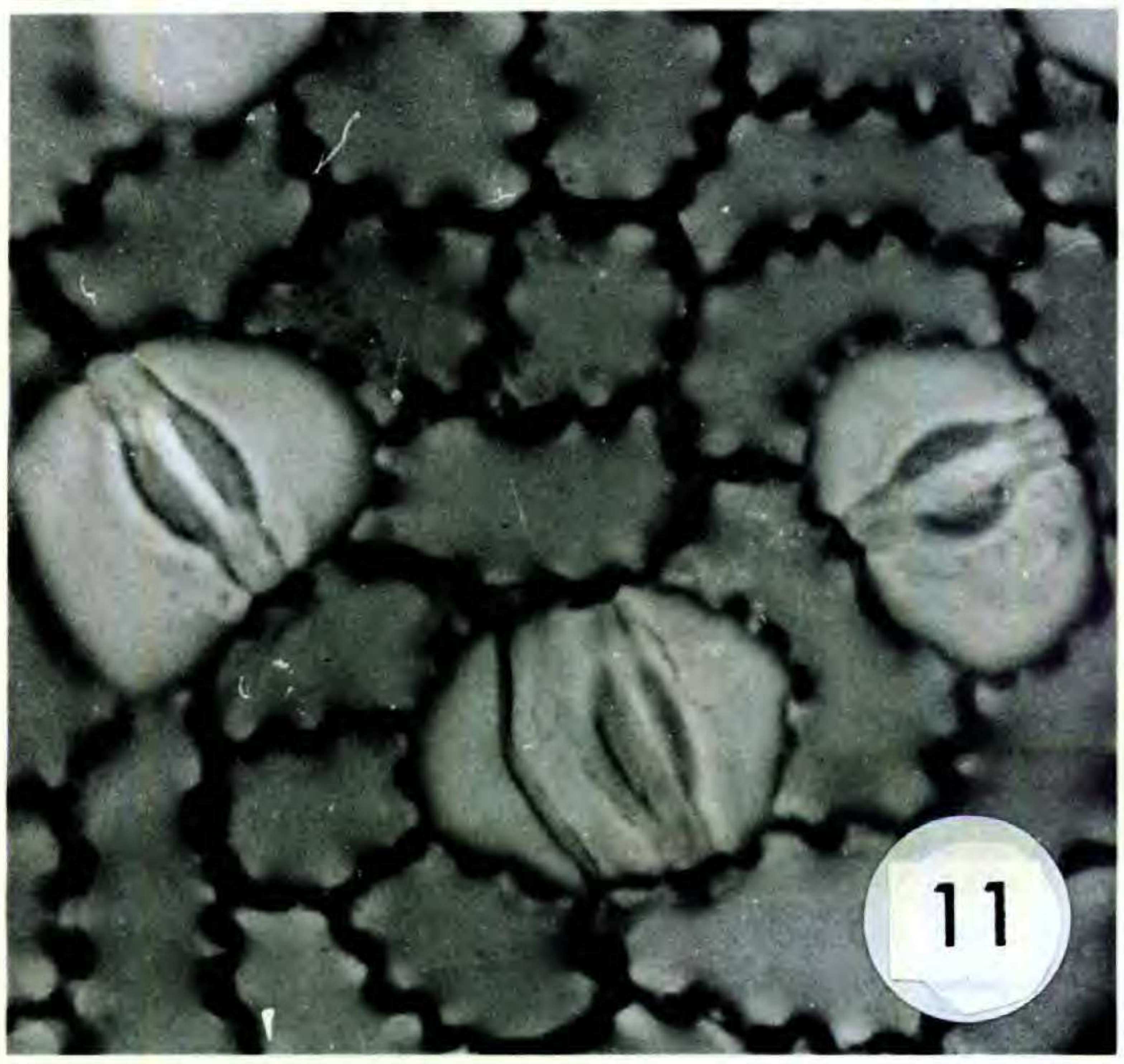

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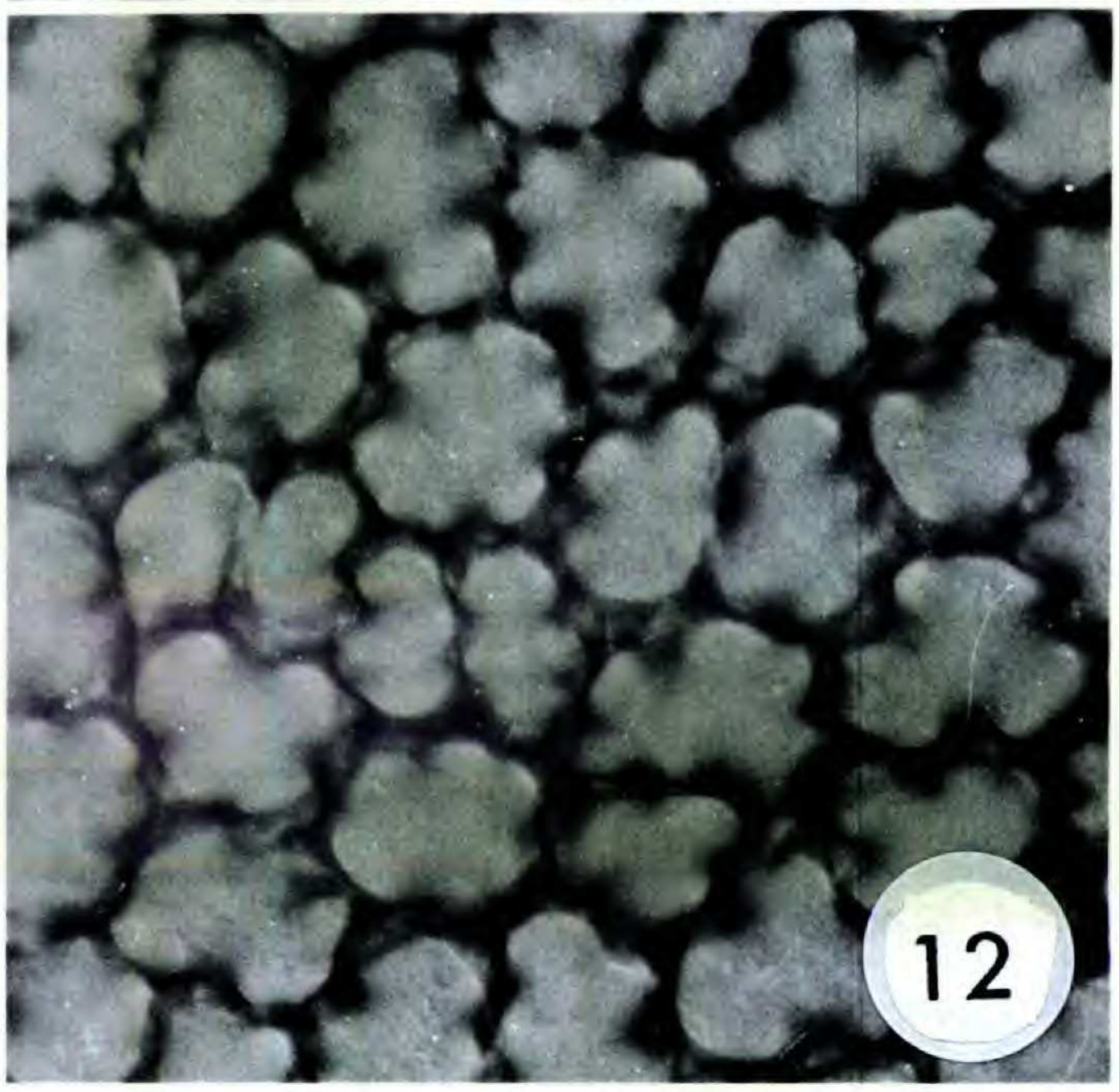

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and truly smooth, but, in practice, artifacts such as remains of epicuticular waxes, stain granules, and bits of adhering cell wall complicate such determinations. These difficulties could have been overcome by rigorous and prolonged chemical treatment, and by observation using SEM; however, one of the major purposes of this study is to develop a character set that is readily usable by general botanists and ecologists, and hence such highly specialized treatment was not employed.

Several separate features of the anticlinal walls can be characterized. The uniformity of thickness is one such character. Anticlinal walls can be regularly uneven (beaded, e.g., Figs. 7, 9), or if sinuous in outline can be thickened in the troughs (buttressed, e.g., Fig. 11), or can be irregularly thickened or smooth, which is the most common state. The straightness of the anticlinal walls is also important, and cuticles are scored for the majority of cells being angular (Fig. 13), regularly or irregularly rounded (Fig. 5), undulate (one trough and peak per wall, e.g., Fig. 18), or sinuous (more than one peak and trough per wall, e.g., Fig. 11). This anticlinal wall shape was placed in a seven category index by Wilkinson (1979), and this alternative method of scoring is also being tried for the global revision, to be followed by analysis to determine the better system. Uniformity of cell size and maximum dimension are also features being included. An example of between-species variation of average cell size can be seen in Figures 7 and 11. Of equal import is the variation apparent in some species in cell size and shape between the adaxial (upper) surface and the abaxial (lower) surface. Relative uniformity of features may be seen in Figures 7 and 8, while variation in shape between surfaces can be seen in Figures 11 and 12, and variation in cell size between surfaces may be seen in Figures 5 and 6.

While the number of subsidiary cells (2) in the Lauraceae and their arrangement (paracytic) are uniform across the family, and all species are hypostomatic, the stomatal complex still provides a rich character set, likely including many of the features that will prove critical in the delimitation of supraspecific taxa. As illustrated in transverse section in Figure 19, and as shown by Hill (1986) in his figure 1, the stomatal complex in the Lauraceae has sunken guard cells with over-arched subsidiary cells. Although the family is truly paracytic, to the non-specialist observing the cuticle in surface view the subsidiary cells appear to be guard cells, and the unspecialized cells in the adjacent row are considered to be subsidiary. Their variable arrangement leads to identifying the subsidiary pattern as virtually nonexistent, or anomocytic.

Possession of uneven-sized subsidiary cells (Fig. 9) has proven common only in certain genera, such as *Beilschmiedia*, and could be of taxonomic utility. By far the most variable feature of the complex, and hence possibly one of the most useful at various levels, is the nature of the two cuticular ledges (Fig. 19). The upper (Fig. 19 UL) protrudes upward and sometimes outward in the stomatal pore, and arises from the lateral walls of the subsidiary cells. In some taxa, the lower ledge (Fig. 19 LL) protrudes between the guard cells (Fig. 19 GC) and the subsidiary cells (Fig. 19 SC), sometimes forming into various shapes including those that look somewhat like butterflies (Figs. 20, 22). An additional taxonomic feature is added to this by the fact that these expanded ledges are variously deciduous.

Some genera, like *Endiandra*, have both ledges visible, although not expanded, and this "double" ledge can thus be used to distinguish this genus in Australia (Christophel & Rowett, in press). Some taxa, including many not found in Australia, have other ledge configurations, and in extreme cases (e.g., Fig. 24, *Aiouea guianensis* Aublet) the entire stomatal complex may be obscured by a thickened ring of cuticle. Finally, some features of cells adjacent to the stomatal complex may also be modified, such as the papillae in *Caryodaphnopsis* (Figs. 1, 3, 4).

The final category of taxonomically useful characters is derived from the presence/absence and features of various specialized cells. In the Lauraceae, all trichomes thus far reported in the litera-

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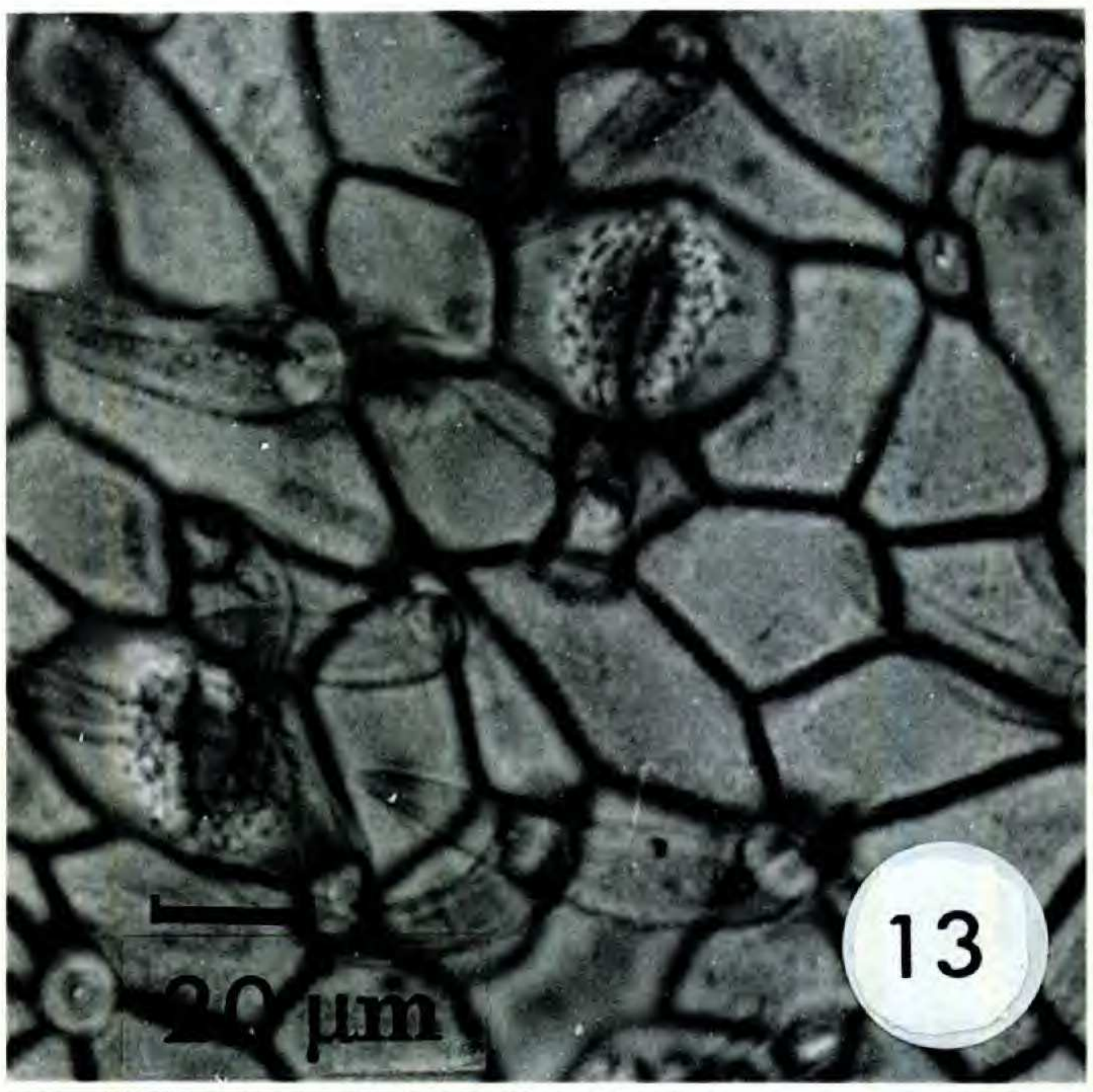
Figures 7–12. —7. Abaxial cuticle of *Beilschmiedia volckii* B. Hyland taken from L650, Botany Department Herbarium, Adelaide University from *B. Gray 3823* housed at QRS; collected in Australia.—8. Adaxial cuticle of *Beilschmiedia volckii.* All information and magnification as per Figure 7.—9. Abaxial cuticle of *Beilschmiedia elliptica* C. White & Francis from L643, Botany Department Herbarium, Adelaide University, from *B. Hyland 4128,* housed at QRS; collected in Australia; magnification as per Figure 7.—10. Adaxial cuticle of *Beilschmiedia elliptica.* All information and magnification as per Figure 7.—11. Abaxial cuticle of *Beilschmiedia obtusifolia* (F. Muell. ex Meissner) F. Muell. from L644, Botany Department Herbarium, Adelaide University, from *B. Gray 1279* housed at QRS; collected in Australia; magnification as per Figure 7.—12. Adaxial cuticle of *Beilschmiedia obtusifolia.* All information and magnification as per Figure 11.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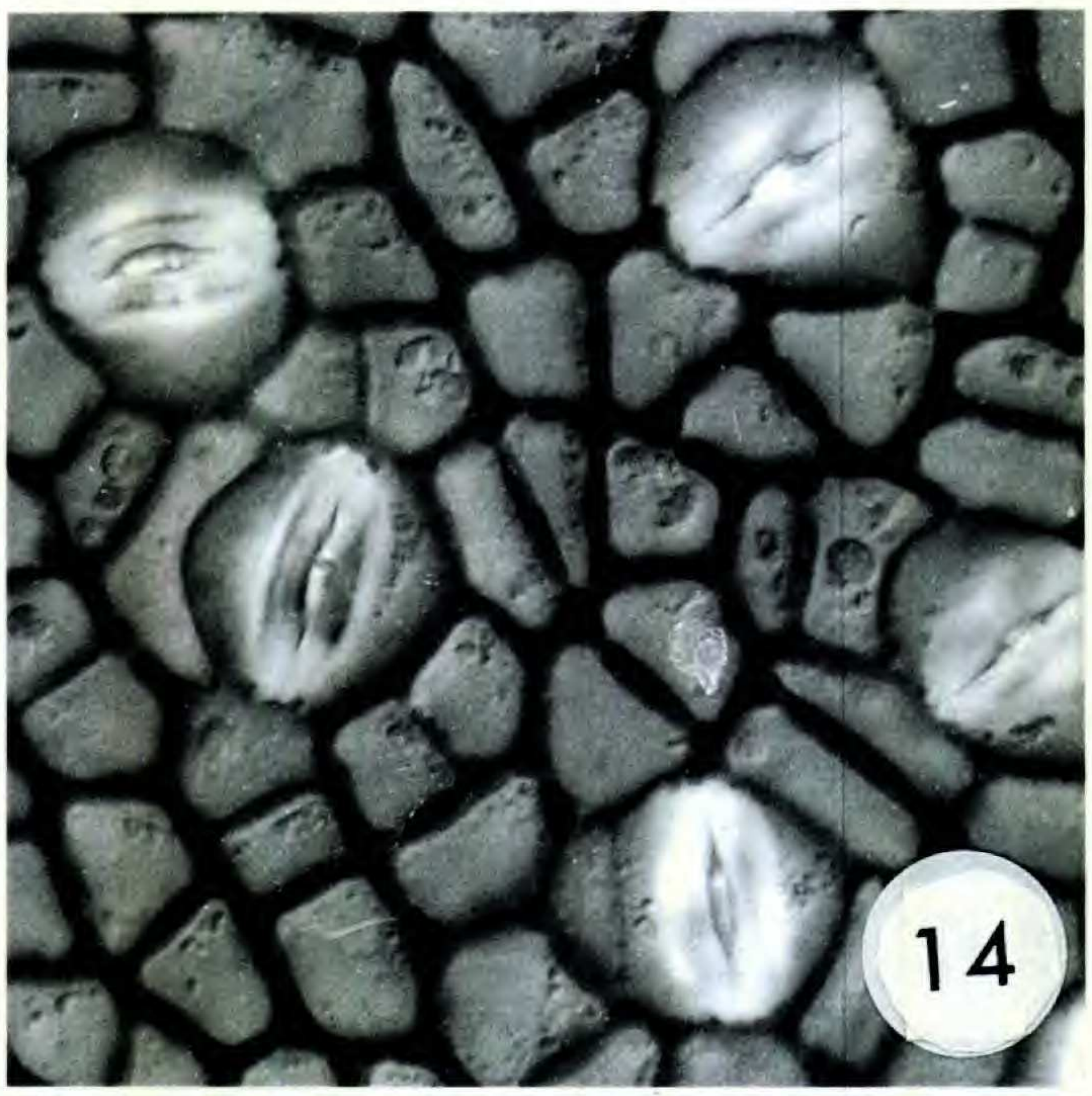
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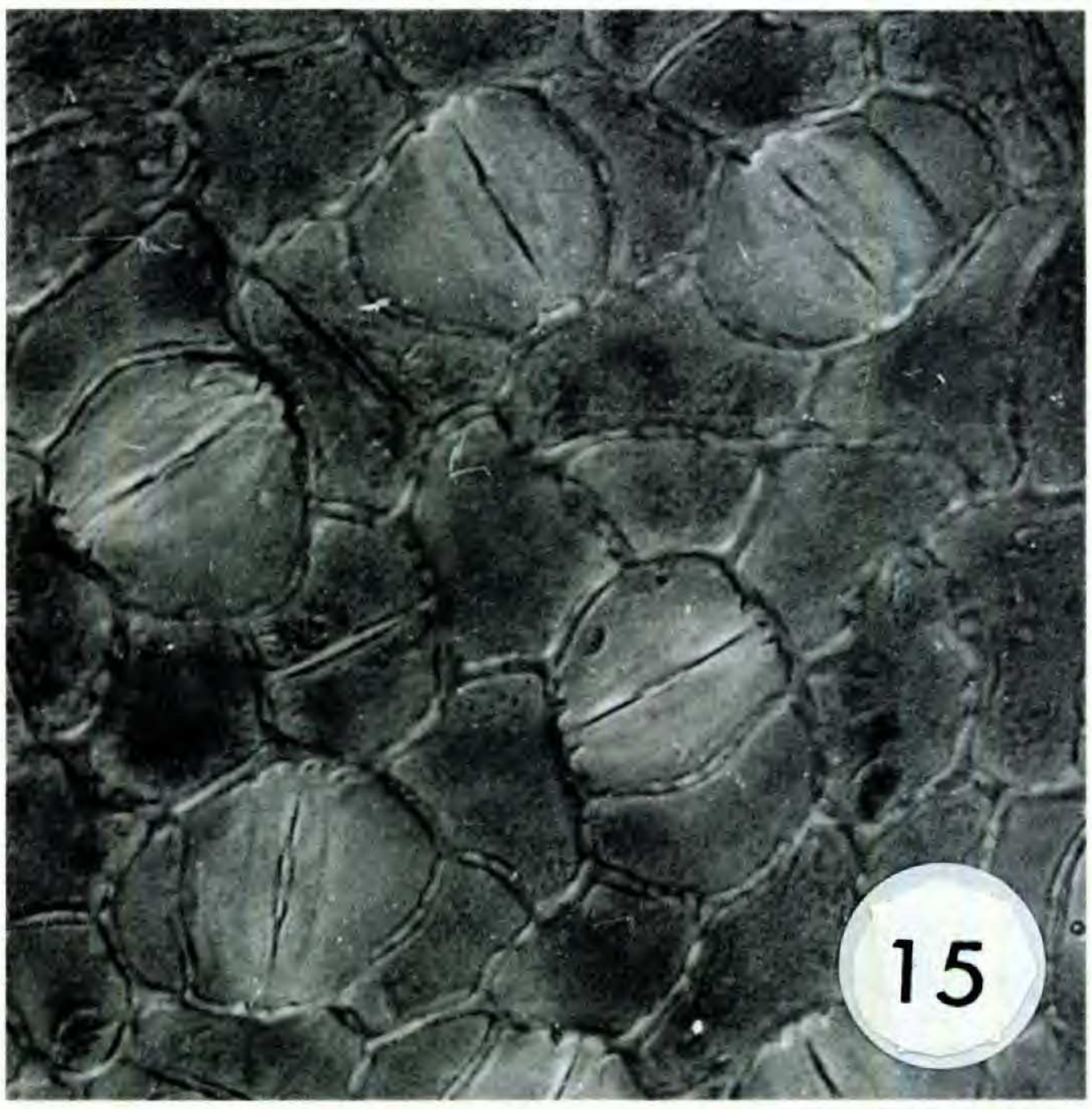
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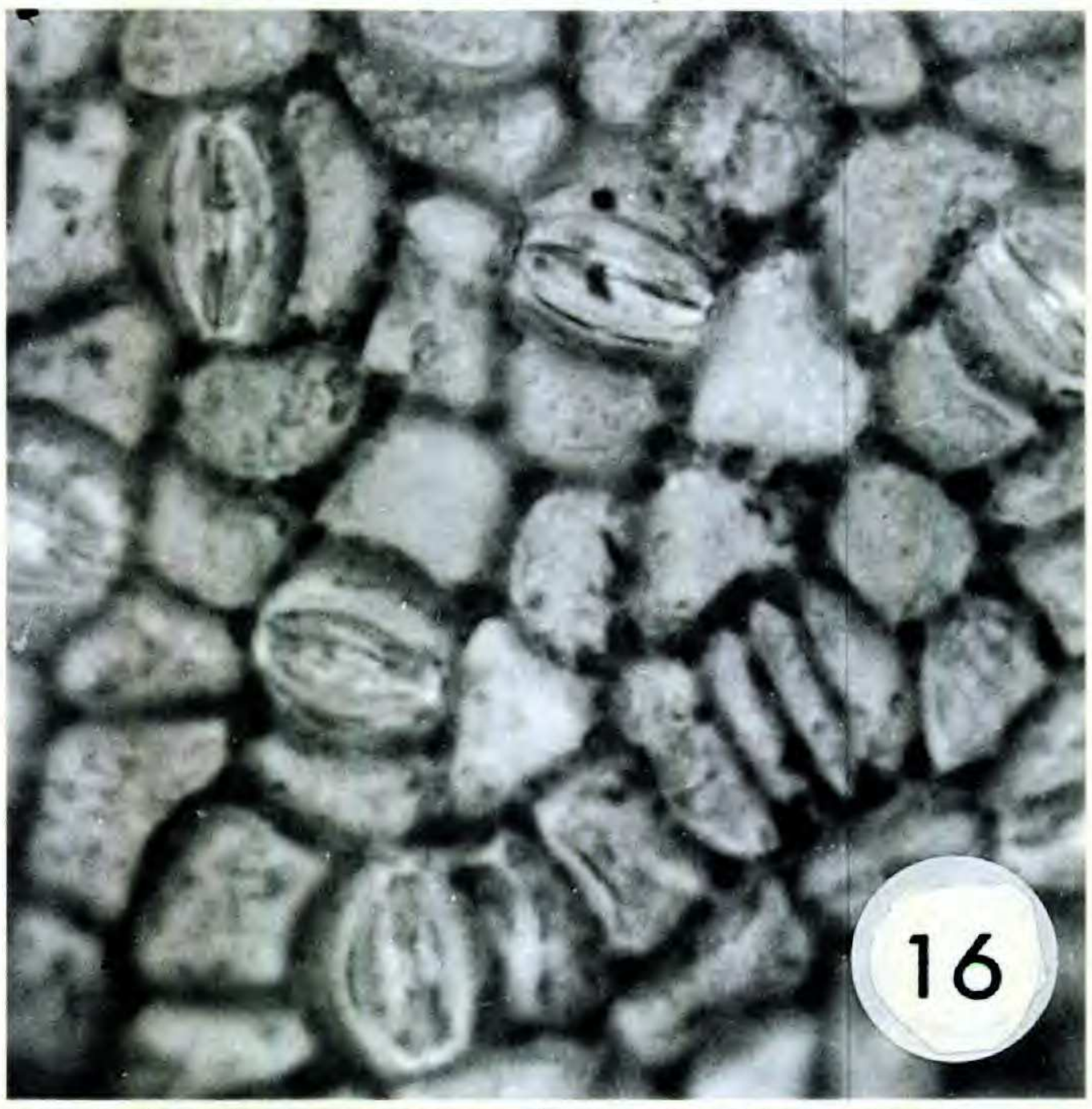
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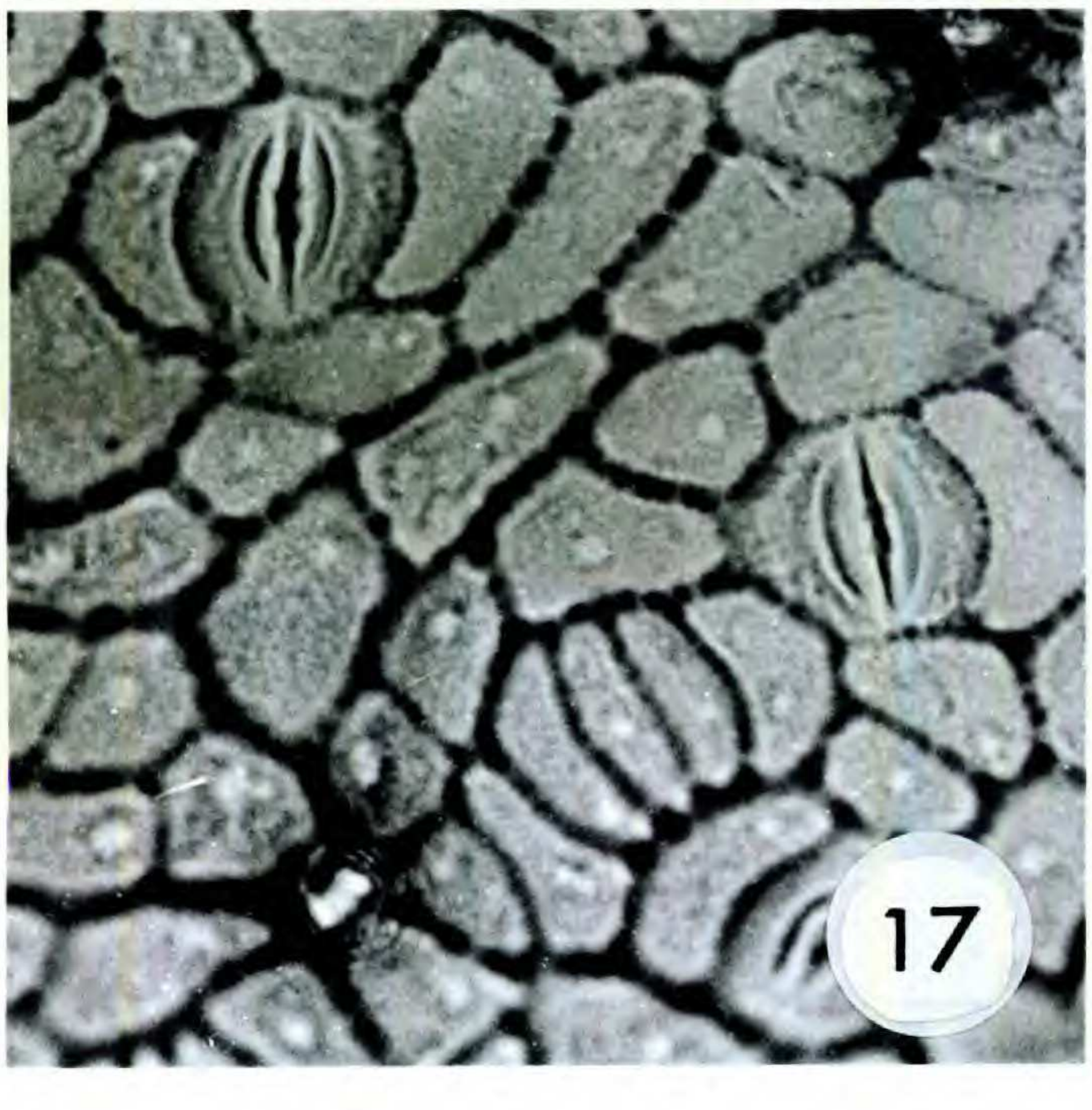
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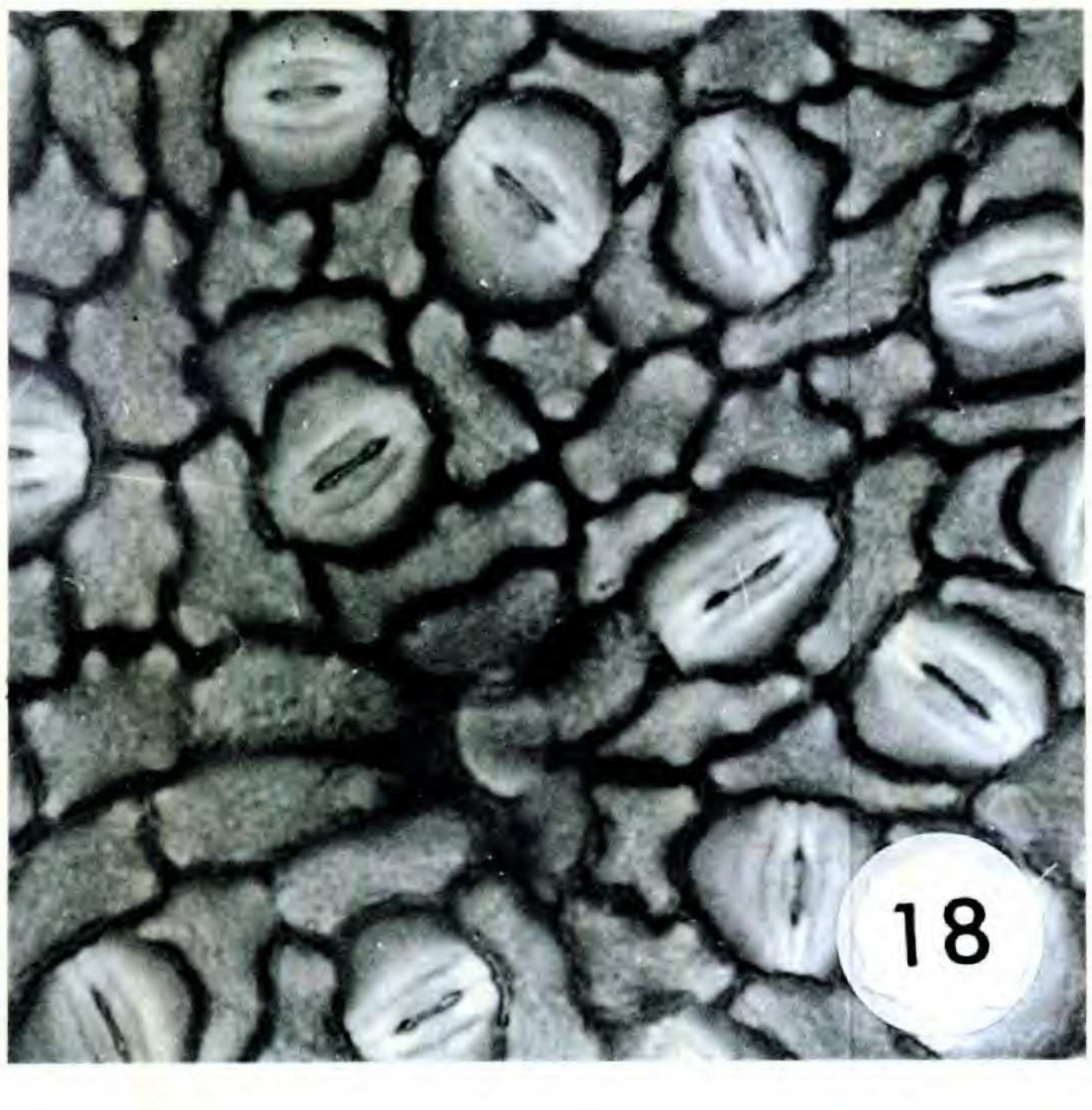
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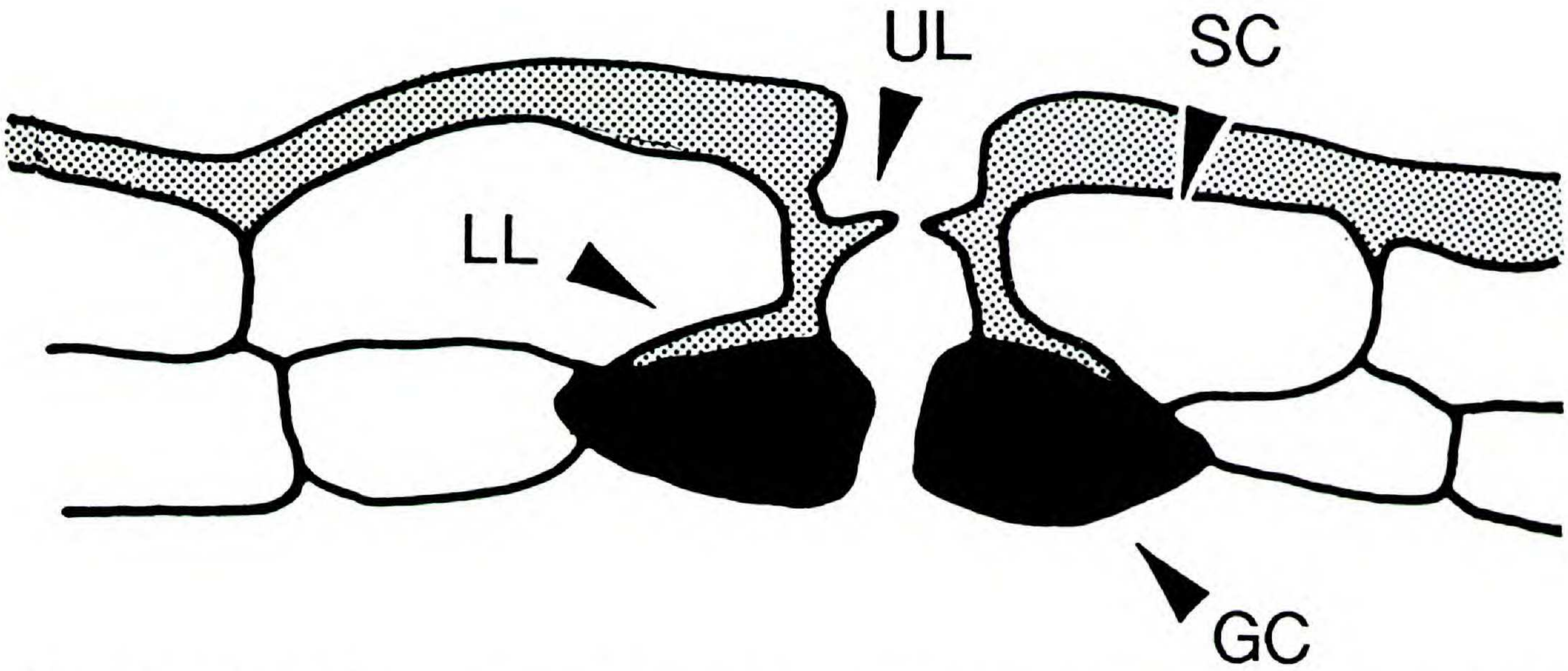

Figure 19. Diagram of transverse section of typical Lauraceae stomatal complex (modified from Hill, 1986, fig. 1). Stippled area shows actual cuticle, as would be seen in a cuticular preparation. Black area represents sunken, embedded guard cells (GC), over-arched by the subsidiary cells (SC). Upper cuticular ledge (UL) and lower cuticular ledge (LL), which vary in prominence across the family, are also shown. Leaf surface toward top in diagram.

ture or observed by the authors have been simple (e.g., *Litsea costalis,* (Nees) Kosterm. Fig. 13). Generally their bases can be characterized as poral, meaning that they consist not of a set of specially modified cells, but of a pore, or hole, at the junction of several non-stomatal cells. In some species, e.g., *Ocotea lentii* Burger, giant trichomes are found that clearly have rings of modified cells surrounding the trichome base. The trichome and its base provide separate suites of characters, as the trichomes are variously deciduous from species to species, and even in those species where they are not deciduous, they can be easily broken in preparation. Obvious features such as trichome density and trichome position relative to veins or areoles are also of potential taxonomic utility.

Some Lauraceae also display regions of thickened cells (5–20) that stain densely and are likely glandular. It should be noted that wound tissue has a similar appearance, but that interpretation can be discounted when the areas are regular in their location across a cuticle slice (and indeed duplicated on additional specimens). The final type of special epidermal structure that has been observed in the Lauraceae is the hydathode. Wilkinson (1979) suggested that structurally there are several types of hydathodes, but within this study the term is restricted to giant stomates. These structures are usually 2–2.5 times the size of normal stomates, and frequently occur over veins. It must be noted that some Lauraceae species have variable-sized stomates, and care must be taken to avoid labeling a large stomate at the extreme of a size range as being a hydathode.

PRELIMINARY RESULTS

When groups of related species or genera are examined for the expression of the above-described characters, some interesting preliminary results can be seen. For example, Christophel and Rowett (in press) noted that *Endiandra* species were charac-

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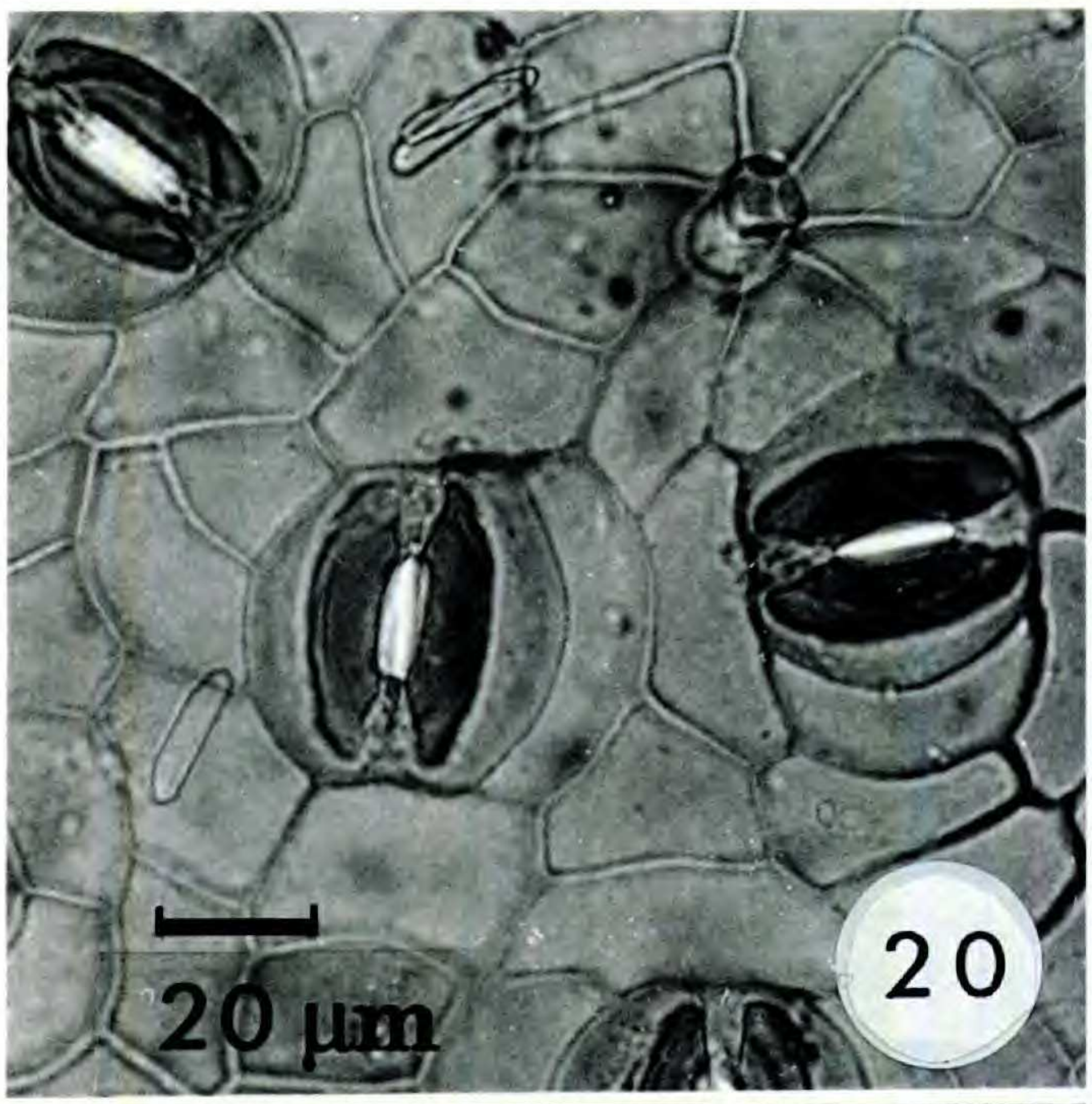
Figures 13–18. —13. Abaxial cuticle of *Litsea costalis* (Nees) Kosterm. Cuticle taken from L190, Botany Department Herbarium, Adelaide University from *B. Hyland 14130* housed at QRS; collected in Sembilan. Note dense simple hairs and poral hair bases.—14. Abaxial cuticle of *Endiandra montana* C. White taken from L691, Botany Department Herbarium, Adelaide University, from *B. Gray 2825* housed in QRS; collected in Australia; magnification as per Figure 13.—15. Abaxial cuticle of *Endiandra glauca* R. Br. Photographed with Nomarski interference contrast microscopy. Cuticle taken from L671, Botany Department Herbarium, Adelaide University from *B. Gray 3211* housed in QRS; collected in Australia; magnification as per Figure 13.—16. Abaxial cuticle of *Endiandra wolfii* B. Hyland. Taken from L705, Botany Department Herbarium, Adelaide University, from *B. Gray 1328* housed at QRS; collected in Australia; magnification as per Figure 13.—17. Abaxial cuticle of *Endiandra sieberi* Nees from L703, Botany Department Herbarium, Adelaide University, from *B. Gray 1504* housed at QRS; collected in Australia; magnification as per Figure 13.—18. Abaxial cuticle of *Endiandra kingiana* Gamble from L528, Botany Department Herbarium, Adelaide University, from *B. Hyland 14146* housed at QRS; collected in Sarawak; magnification as per Figure 13.



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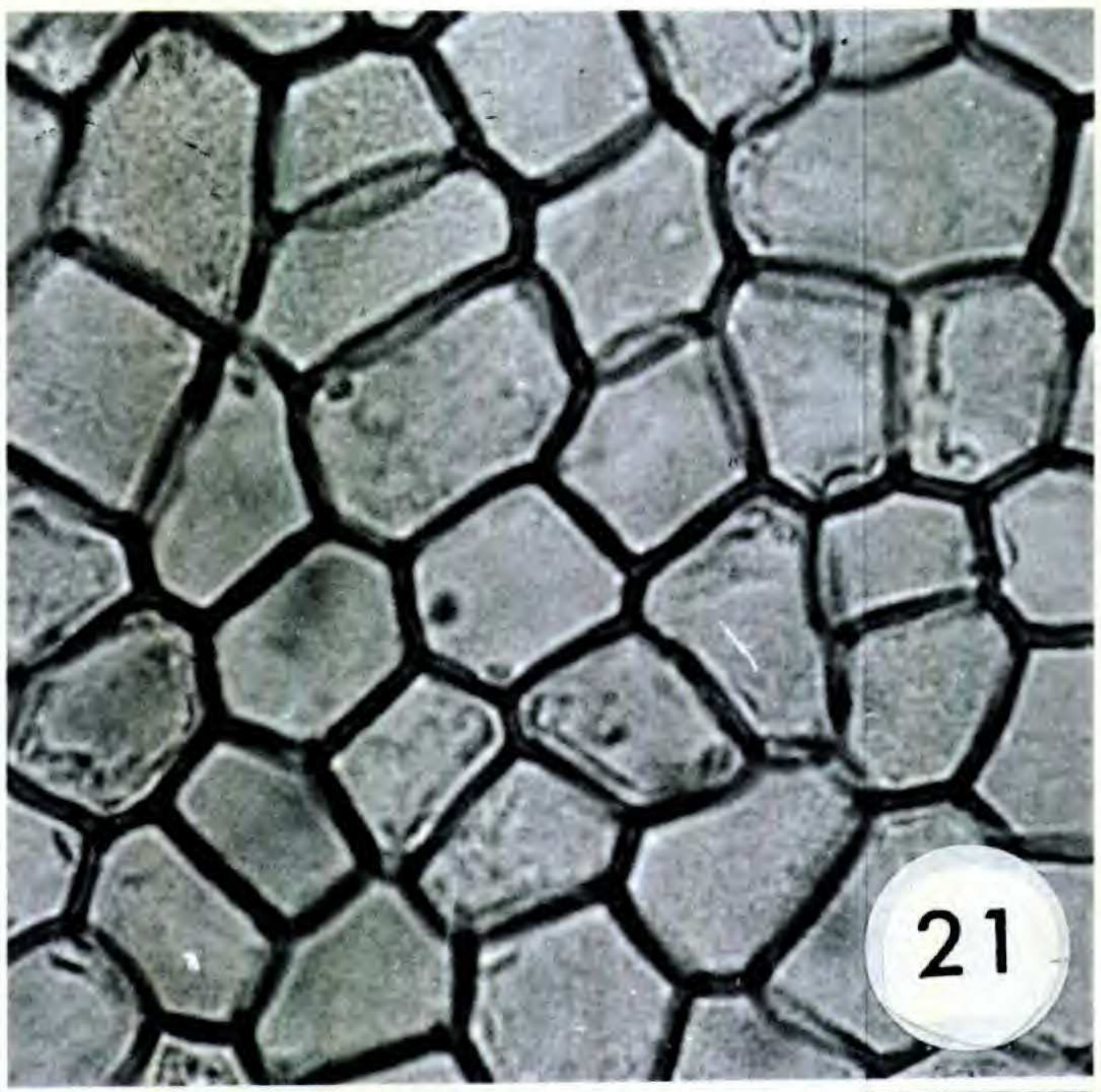

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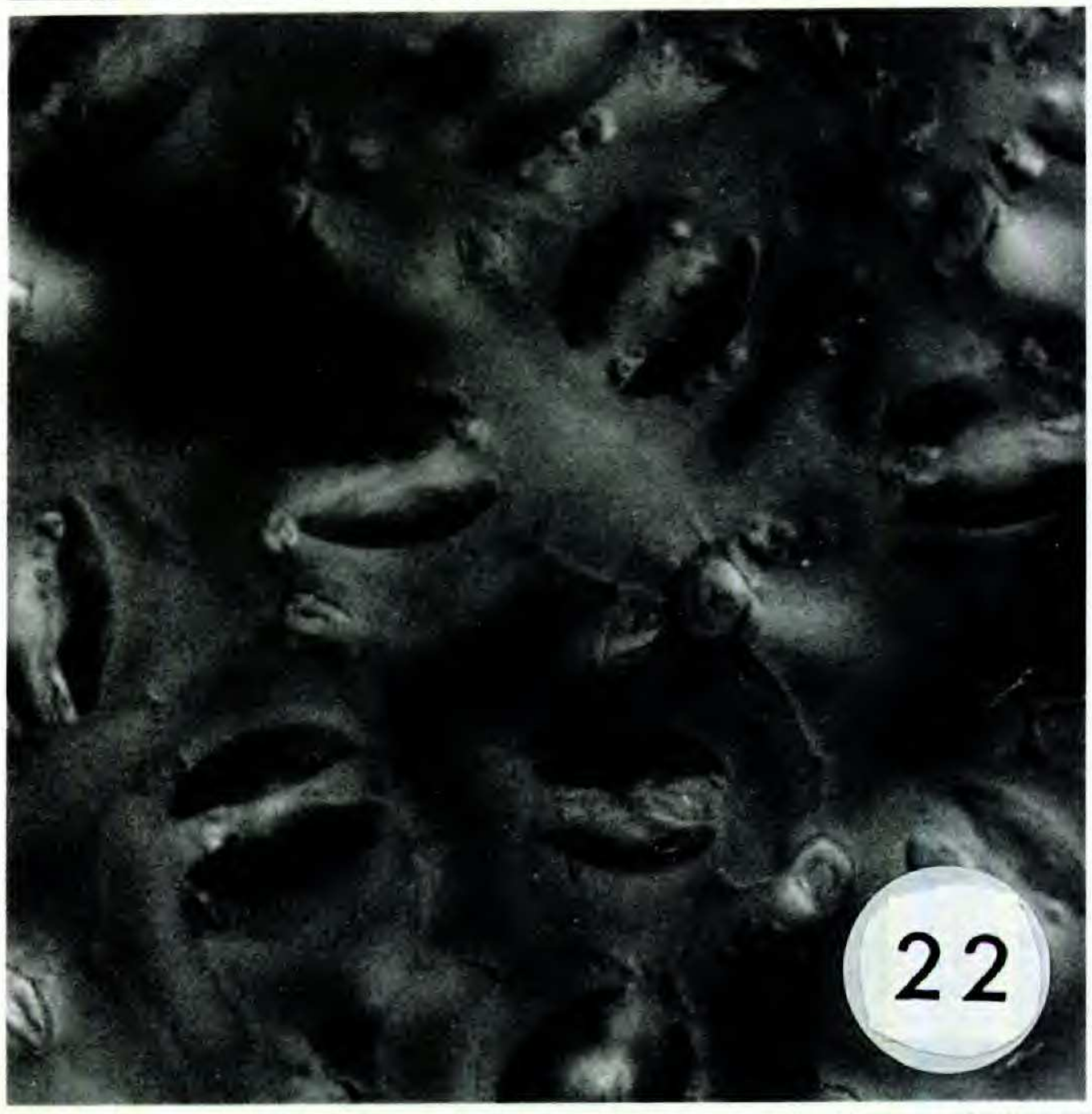

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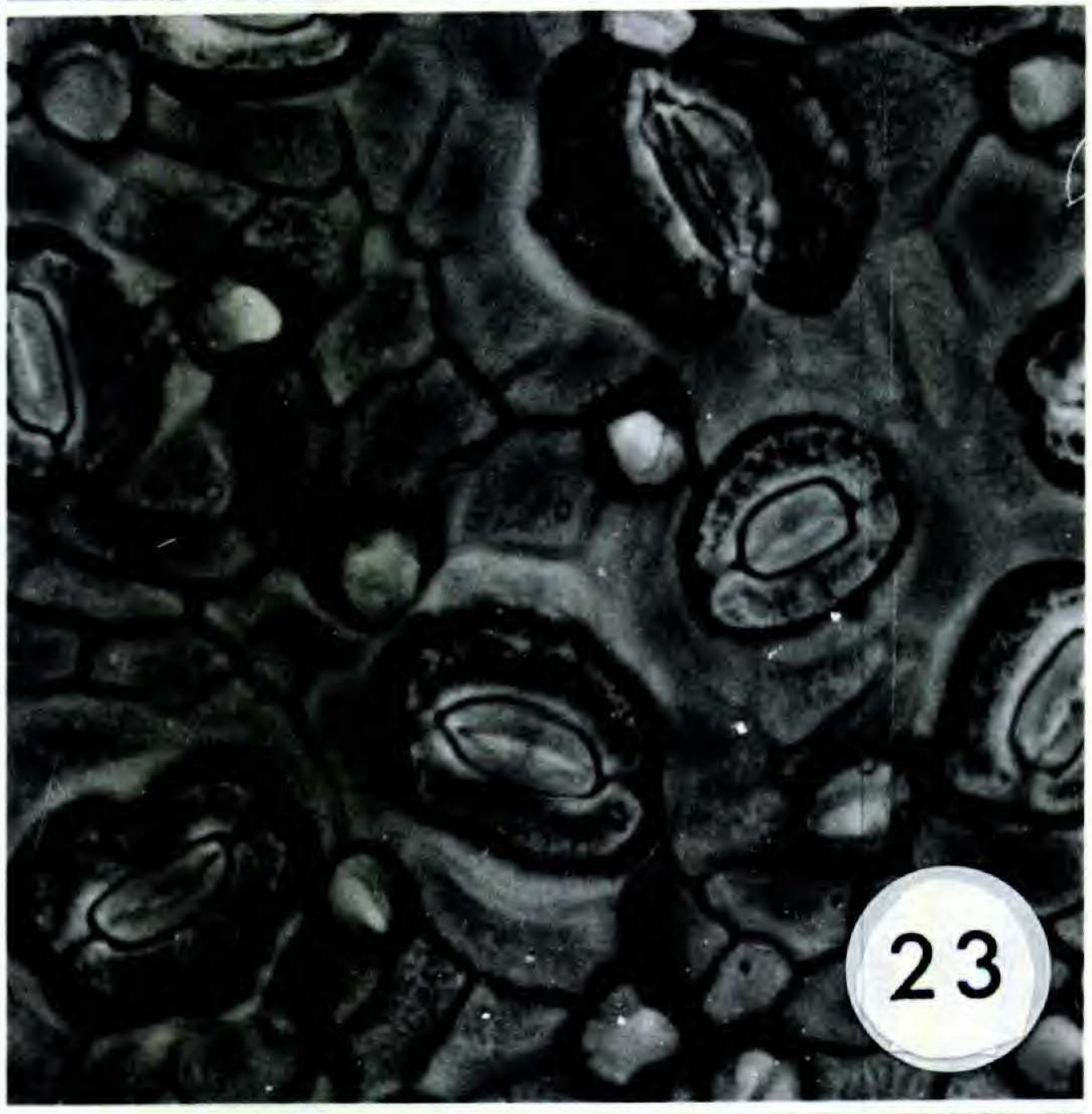

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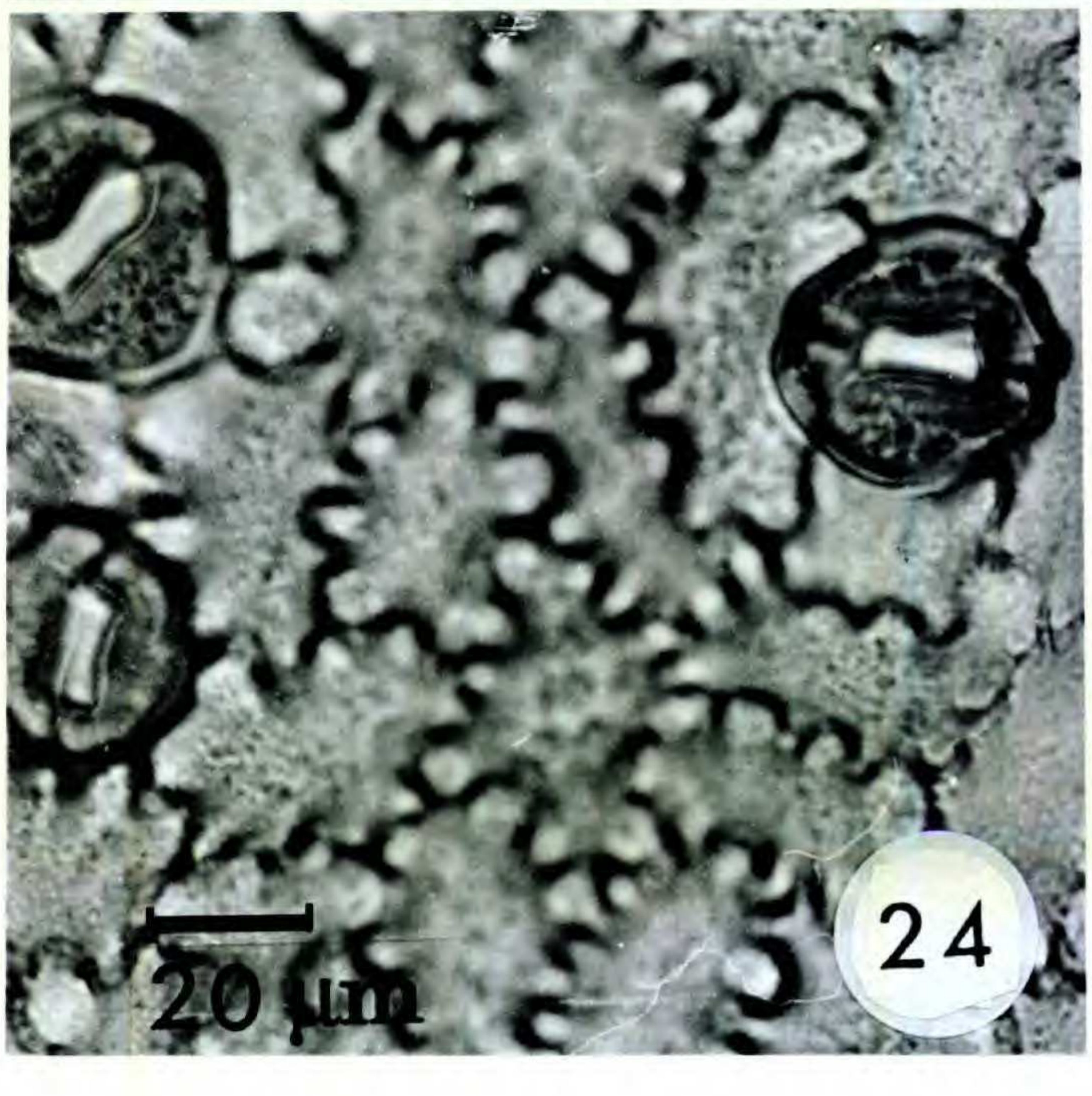

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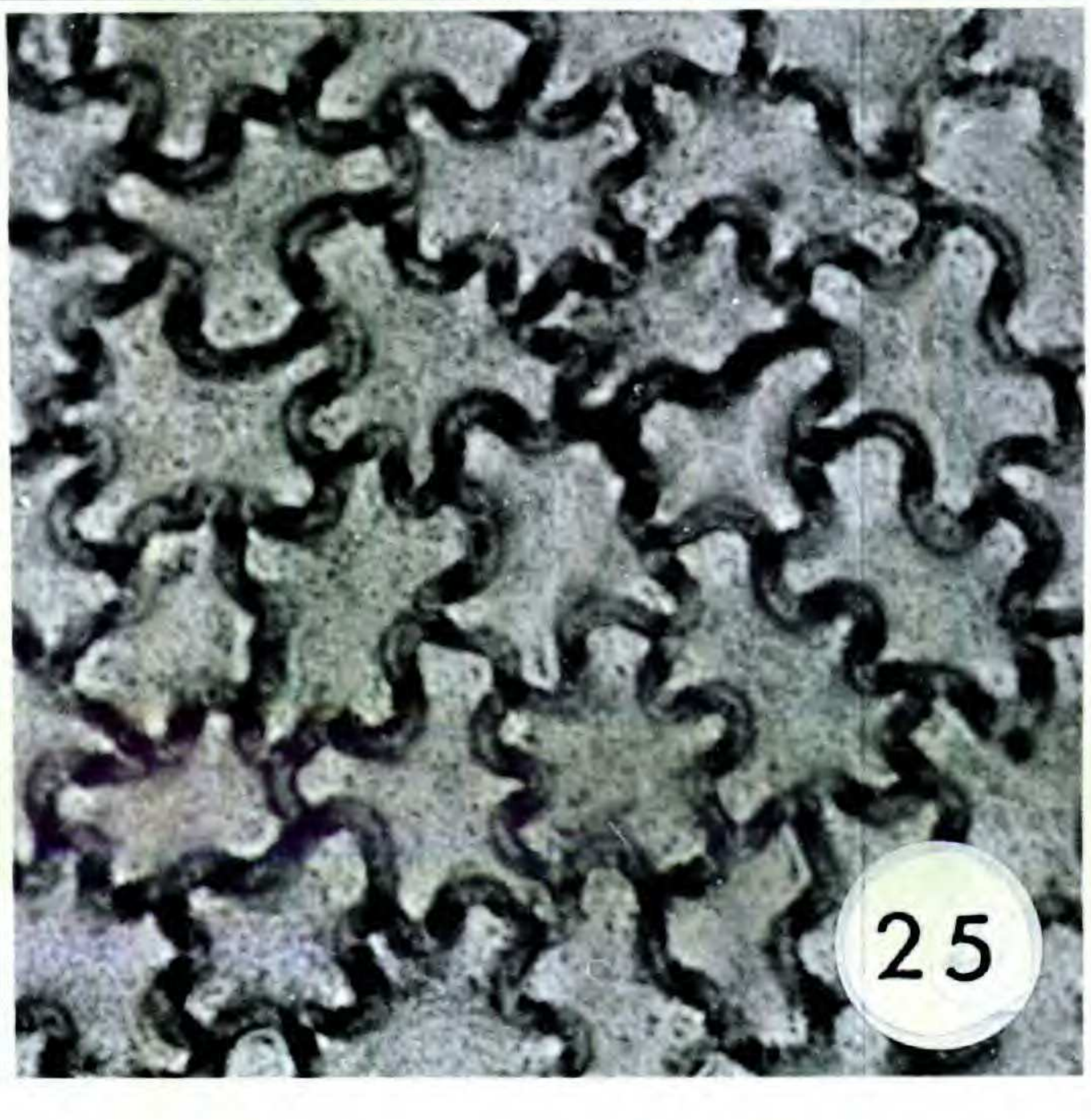

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terized in Australia by having relatively straight, thin, double stomatal ledges. These may be observed in Figures 14, 15, 16, and 17, where it can be seen that a thin, inner (upper) ledge is visible just inside the stomatal pore, and another parallel set of ledges (lower) can be seen outside of these, separated by approximately one-third of the subsidiary cell, and parallel to its long axis. While the other three Australian species illustrated (Figs. 14, 16, 17) are photographed with normal transmitted light, *Endiandra sieberi* Nees (Fig. 15) was photographed with interference contrast (Nomarski) microscopy, to compensate for unsatisfactory staining of that particular specimen. Not only does this clearly show the pairs of ledges, but the faint outlines suggest the position of the sunken guard cells may also be seen. *Beilschmiedia,* the sister genus to *Endiandra,* lacks these straight, double ledges. While straight, double ledges appear a good descriptor for *Endiandra,* it should be noted that other features are variable. For example, the anticlinal walls of *Endiandra montana* C. White, *E. wolfii* B. Hyland, and *E. glauca* R. Br. are straight, whereas *E. sieberi* Nees has irregularly rounded to undulate anticlinal walls.

A measure of the success of this character must be its effectiveness on extra-Australian species. To date 20 such species have been examined, and all show the double ledges. Some (e.g., *Endiandra kingiana* Gamble from Sarawak—Fig. 18) even illustrate the feature more clearly than the Australian species.

Beilschmiedia is considered to be closely related to *Endiandra* by most authorities (e.g., Hyland, 1989; Kostermans, 1957; Rohwer, 1993). In general, it can be separated easily by the fact that its species normally possess nine fertile anthers. However, just as in *Endiandra,* in Australia a group of *Beilschmiedia* species (4) possess only six anthers. While this would qualify them for membership in Allen's *Brassiodendron,* Hyland (1989) has placed them in *Beilschmiedia* based on a suite of other features.

Christophel and Rowett (in press) were able to separate *Endiandra* and *Beilschmiedia* in Australia quite easily in that no species of *Beilschmiedia* had the nearly straight, double ledge that *Endiandra* stomates possess. *Beilschmiedia* itself is characterized by all species having some degree of uneven anticlinal wall thickening (buttressing or beading) (Figs. 7, 9, 11). Some species, such as *Beilschmiedia obtusifolia* (F. Muell. ex Meissner) F. Muell. (Figs. 11, 12), show a marked degree of difference in the degree of thickening between the adaxial and abaxial walls. This feature, however, is not restricted to *Beilschmiedia,* as some *Endiandra* species show anticlinal wall thickening.

In addition, all Australian species appear to have some degree of unevenness in the size of the two subsidiary cells (e.g., *Beilschmiedia elliptica* C. White & Francis, Fig. 8). This is not as reliable a feature for generalized identification, however, as in many species it is not visible on stomates in some regions; hence a random leaf piece might not show them.

Cuticular analysis can also be used on species groups, with potential biogeographical significance. One of the most obvious disjunctions in the family occurs in *Caryodaphnopsis,* which has species recorded from South America and also from Southeast Asia. Examination of the cuticles of *Caryodaphnopsis* species shows not only some unique features, but also highlights the fact that both geographic groups possess them. While the adaxial (upper) cuticular surface of *Caryodaphnopsis* species usually has smooth to granular periclinal walls (Fig. 2), the abaxial surface invariably has papillae present to some extent on the periclinal walls. The density of the papillae varies from species to species, but in all species examined papillae are clearly present on the cells surrounding the stomatal complex, and even over-arch it to a degree. This feature can be

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Figures 20–25. —20. Abaxial cuticular surface of *Rhodostemonodaphne grandis* (Mez) Rohwer showing butterfly-shaped lower cuticular ledge (dark stained). Cuticle from specimen L104, Botany Department Herbarium, Adelaide University, from *Mori 20904* at MO; collected in French Guiana.—21. Adaxial cuticular surface of *Rhodostemonodaphne grandis*. Specimen citation and scale bar as per Figure 20.—22. Abaxial cuticular surface of *Rhodostemonodaphne kunthiana* (Nees) Rohwer showing butterfly-shaped lower cuticular ledges. Photographed with Nomarski interference contrast microscopy; prepared from L106, Botany Department Herbarium, Adelaide University. Originally from *Zak 4452* at MO; collected in Ecuador; magnification as per Figure 20.—23. Abaxial cuticle of *Cinnamomum sellovianum* (Nees & Mart.) Kosterm. showing cuticular collar surrounding stomatal apparatus; taken from L124, Botany Department Herbarium of Adelaide University. Prepared from *Harley 25261* at MO; collected in Brazil; magnification as per Figure 20.—24. Abaxial cuticle of *Aiouea guianensis* Aublet from L113, Botany Department Herbarium, Adelaide University, from *Jensen Jacobs 1986* at MO; collected in Guyana.—25. Adaxial cuticle of *Aiouea guianensis.* All information and magnification as per Figure 24.

seen in the three species illustrated for this paper (Figs. 1, 3, 4); two of these (*Caryodaphnopis henrii* A. Shaw and *C. tonkinense* (Lec.) A. Shaw) were collected in China and the third, an as-yet-undescribed species, occurs in Peru.

DISCUSSION

The use of cuticular features for various taxonomic delimitations has been successful in various diverse groups (e.g., Araucariaceae—Stockey & Ko, 1986; Myrtaceae—Christophel & Lys, 1986). It has also proven satisfactory for family identification (Hill, 1986) and local generic differentiation (Ow et al., 1992) within the Lauraceae. Preliminary investigations have now shown in this paper that, based on genera such as *Endiandra, Beilschmiedia,* and *Caryodaphnopsis,* the use of a suite of cuticular characters applied to a global range of genera and species has great promise.

Concerning the examination of *Endiandra,* of particular interest among the Australian species are the two that have six anthers (all other species in the genus possess three anthers). Hyland (1989) commented on them and noted that while some workers (e.g., Allen, 1942) placed them in a separate genus, *Brassiodendron,* he (Hyland) considered anther number by itself to be a non-definitive character, and included the two species in *Endiandra* based on a majority of other features. *Endiandra montana* C. White (Fig. 14) is one of those six-anthered species, and is shown to clearly belong with the other *Endiandra* species because of the double ledge cuticle feature. The other six-anthered species, while not illustrated here, also has the double ledge, and hence supports Hyland's placement in *Endiandra.* It should be noted that no attempt is made here to infer that the double ledge character is more taxonomically important than anther number, but it does support Hyland's placement of those species in *Endiandra,* which was based on a suite of morphological characters.

The four species of *Beilschmiedia* in Australia that possess six anthers have been examined, and show no obvious differences from the remaining species in the genus. This can be seen by comparing one of those four, *Beilschmiedia volckii* B. Hyland (Figs. 7, 8), to the nine-anthered species *Beilschmiedia obtusifolia* (F. Muell. ex Meissner) F. Muell. and *B. elliptica* C. White & Francis (Figs. 9, 10, 11, 12). Perhaps of even more significance is the fact that they are clearly distinct from the six-anthered species of *Endiandra* in their stomatal ledge structure. The few species of extra-Australian *Beilschmiedia* examined thus far have agreed with the features found by Christophel and Rowett (1995) for that genus in Australia.

Following from the results of the study of *Caryodaphnopsis* cuticles, it should be noted that in the Lauraceae, papillae are not restricted to that genus. For example, they can be found in a few species of *Beilschmiedia* and are reported to be common in *Aniba* (Kubitzki & Renner, 1982). However, it is the similarity of the structure of the papillae, and not only their presence, that supports the placement of these disjunct species of *Caryodaphnopsis* in the single genus.

The exclusive use of cuticle characters within the family is not without some problems, or at least challenges, however. Christophel and Rowett (in press) have shown that within Australian species of *Cryptocarya,* good generic delimiting features are not obvious. Examination of approximately 20 additional species from this genus occurring outside of Australia has not solved this difficulty, although it has become clear that the decision to sink *Ravensara* from Madagascar into *Cryptocarya* s.l. is a good one, based on epidermal similarities.

Another group that should prove challenging is *Cinnamomum.* It can be seen that South American species of this genus have distinctive cuticular signatures (Fig. 23) and that they are clearly different from the *Cinnamomum* species of Australia figured by Christophel and Rowett (in press). Interestingly, the American *Cinnamomum* species had been placed until recently in *Phoebe* (Rohwer, 1993). Asian species of the genus, including the type species, form a third distinctive group, and further study may well lead to the conclusion that the group is not natural (and is perhaps polyphyletic) as it is currently defined.

The tripli-nerved, or acrodromous venation pattern is common in both *Crypotcarya* and *Cinnamomum.* However, no positive correlation has been found between groups of species possessing that character and those sharing common cuticular features. Basic venation characters are nonetheless being initially included in the global revision in order to test them on the full spectrum of species in the family.

Hyland (1989) suggested that the generic complex of *Litsea, Neolitsea,* and *Lindera* is also in need of further detailed study. Christophel and Rowett (in press) had little difficulty separating the three genera based entirely on Australian material, but those species represent only a small proportion of the total for the three genera, and the lack of single outstanding features to separate them, similar to the double ledges of *Endiandra,* suggests that the group could provide a challenge on a global level.

Finally, some of the largest genera (e.g., *Ocotea* and *Nectandra*) occur in South America, and that material has been the least thoroughly studied by us to date. A strong possibility exists that both of these genera are currently unnatural (H. van der Werff, MO, pers. comm.) and hence much work needs to be done here. Preliminary observations on material from various genera in South America, however, suggest that some features that are highly diagnostic in Australian material (e.g., the butterfly-shaped cuticular ledges prominent in *Cryptocarya* and to a lesser extent, *Cinnamomum*) are in fact widespread in other geographic regions and in other currently prescribed taxa. The potential utility of such characters will only be assessable once thorough studies of the material have been made.

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